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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US99/02128 <b>(22) International Filing Date:</b> 29 January 1999 (29.01.99)  <b>(30) Priority Data:</b> 60/073,043 29 January 1998 (29.01.98) US  <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application</b> US 60/073,043 (CIP) Filed on 29 January 1998 (29.01.98)  <b>(71) Applicant (for all designated States except US):</b> THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK [US/US]; West 116th Street and Broadway, New York, NY 10027 (US).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> CHRISTIANO, Angela, M. [US/US]; 70 Jeffrey Circle, Dayton, NJ 08810 (US).  <b>(74) Agent:</b> WHITE, John, P.; Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY 10036 (US).		<b>(81) Designated States:</b> AU, CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> HUMAN HAIRLESS GENE, PROTEIN AND USES THEREOF  <b>(57) Abstract</b>  The present invention provides an isolated nucleic acid which encodes a wildtype or mutant human hairless protein. The present invention further provides an isolated wildtype or mutant human hairless protein. In addition, the present invention provides methods of isolating a nucleic acid encoding a wildtype human hairless-related protein in a sample containing nucleic acid, methods for identifying a compound which is capable of enhancing or inhibiting expression of a human hairless protein, methods for identifying a binding compound which is capable of forming a complex with a human hairless protein, and methods for identifying an inhibitory compound which is capable of interfering the capacity of a human hairless protein to form a complex with the binding compound. The invention also provides a transgenic animal and pharmaceutical compositions and methods for treating a human hairless condition.		

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HUMAN HAIRLESS GENE, PROTEIN AND USES THEREOF

5 This application is a continuation-in-part of Provisional Application No. 60/073,043, filed January 29, 1998, the contents of which are hereby incorporated by reference.

10 Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

15

Background of the Invention

Human Hair Follicle Development. The human hair follicle is a dynamic structure which generates hair through a complex and highly regulated cycle of growth and remodeling. Hardy, 1992, *Trends Genet.* 8:159; Rosenquist and Martin, 1996, *Dev. Dynamics* 205:379. During embryogenesis, the follicle is initially formed as a downgrowth of the overlying surface ectoderm in response to an initial dermal message to the ectoderm dictating the formation of an appendage. Next, it has been speculated that an epidermal message passes from the epithelial cells in the follicle bud to an underlying cluster of dermal mesenchymal cells, known as dermal papilla cells. The dermal papilla functions as the signaling center which plays a central role in regulating the subsequent development and activity of the hair follicle. Finally, a second dermal message is transmitted from the dermal papilla cells to the overlying epithelial cells of the hair plug, now known as the "hair matrix," stimulating them to divide rapidly, to form the mature hair follicle. *Id.*

As the follicle develops, morphologically, it appears as a bulbous structure with a rounded base (the hair bulb) from

which a long neck extends upward that connects it to the skin surface. The hair bulb surrounds the underlying dermal papilla, and contains a highly proliferative cell population, the hair matrix, whose progeny are gradually displaced upward toward the surface. As they traverse the keratogenous zone at the top of the hair bulb at the base of the neck, the cells begin to differentiate into at least six different cell types that are organized in concentric layers. The three innermost layers form the medullary, cortical and cuticular layers of the emerging hair, and the three sequentially more peripheral outer layers form the inner root sheath, which extends part of the distance up and is shed into the neck of the follicle. As the hair elongates, it passes through the skin surface, through the pilary canal. *Id.*

Hair Growth Cycle. Hair growth is typically described as having three distinct phases. In the first phase, known as anagen, the follicle is generated and a new hair grows. During the second stage, known as catagen, the follicle enters the stage where elongation ceases and the follicle regresses because the matrix cells stop proliferating. At this "catagen" stage, the lower, transient, half of the follicle is eliminated as a result of terminal differentiation and keratinization, and programmed cell death. Rosenquist and Martin, 1996, *Dev. Dynamics* 205:379. Also during catagen, although the dermal papilla remains intact, it undergoes several remodeling events, including degradation of the elaborate extracellular matrix which is deposited during anagen. At the close of catagen, the hair is only loosely anchored in a matrix of keratin, with the dermal papilla located just below. The catagen stage occurs at a genetically predetermined time which is specific for each hair type in a species. The third stage, known as telogen, is characterized by the follicle entering a quiescent phase, during which the hair is usually shed.

When a new hair cycle is initiated, it is thought that a signal from the dermal papilla stimulates the stem cells, which are thought to reside in the permanent portion of the follicle, to undergo a phase of downward proliferation and  
5 genesis of a new bulbous base containing matrix cells which then surround the dermal papilla. As the new anagen stage progresses, these hair matrix cells produce a new hair, and the cycle begins again. Each follicle appears to be under  
10 completely asynchronous control, resulting in a continuum of follicles in anagen, catagen, and telogen phases in adjacent follicles, leading to a relatively homogeneous, uniform hair or coat distribution. Hardy, 1992, *Trends Genet.* 8:159; Rosenquist and Martin, 1996, *Dev. Dynamics* 205:379.

15 Despite this descriptive understanding of the hair cycle, currently very little is known about the molecular control of the signals that regulate progression through this cycle. Notwithstanding this lack of knowledge with respect to the  
20 molecular control of the signals responsible for hair growth, it is clear that at least some potentially influential regulatory molecules may play a role. For example, a knock-out mouse with targeted ablation of the fibroblast growth factor 5 (FGF5) gene provides evidence  
25 that FGF5 is an inhibitor of hair elongation. Specifically, it has been observed that the knock-out mouse has an increase in hair length due to an increase in the time that follicles remain in anagen. The FGF5 gene was also deleted in the naturally occurring mouse model, *angora*, to determine  
30 the effect FGF5 expression on hair growth and development. Hebert, et al., 1994, *Cell* 78:1017.

Another member of the FGF family, FGF7 or keratinocyte growth factor, was disrupted by gene targeting, and the  
35 resultant mouse had hair with a greasy matted appearance, similar in phenotype to the *rough* mouse. Guo, et al., 1996,

Genes & Devel. 10:165. A transgenic mouse was engineered which disrupted the spatial and temporal expression of the lymphoid enhancer factor 1 (LEF1) gene, a transcription factor that binds to the promoter region of 13 out of 13 published hair keratin promoters. It was shown that disruption of this potential master regulator of hair keratin transcription led to defects in the positioning and angling of the hair follicles, a process previously assumed, though never proven, to be under mesenchymal control. Zhou, et al., 1995, *Genes & Devel.* 9:700. More recently, a mutation in the mouse desmoglein 3 gene (*dsg3*) was found to be the cause of the naturally occurring mouse, *balding*. Koch, et al., 1997, *J Cell Biol.* 137:1091. The congenital alopecia and athymia in the nude mouse results from mutations in the *whn* gene (winged-helix-nude, *Hfh 11<sup>nu</sup>*), which encodes a forkhead/winged helix transcription factor with restricted expression in thymus and skin. Nehls, et al., 1994, *Nature* 372:103; Segre, et al., 1995, *Genomics* 28:549; Huth, et al. 1997, *Immunogenetics* 45:282; Hofmann, et al., 1998, *Genomics* 52:197; Schorpp, et al., 1997, *Immunogenetics* 46:509. In addition to the complexity of the signaling pathways, in sheep, there are over 100 distinct structural proteins synthesized by the hair cortex and cuticle cells which produce the keratinized structure of the wool fiber. Hardy, 1992, *Trends Genet.* 8:159. Despite these examples of recent progress in murine models, the control and molecular complexity of the hair follicle and its cyclic progressions in humans is only beginning to be understood.

The Alopecias: The Hereditary Nature Of Hair Loss. There are several forms of hereditary human hair loss, known collectively as alopecias, which may represent a dysregulation of the hair cycle. The molecular basis of the alopecias, however, is unknown. Rook and Dawber, 1991, *Diseases of the Hair and Scalp* (Blackwell Press, Oxford, UK,



ed. 2,) pp. 136-166. The most common form of hair loss, known as androgenetic alopecia (male pattern baldness) is believed by some to represent a dominantly inherited allele affecting 80% of the population. Bergfeld, 1995, *Am. J. Med.*

5 98:95S-98S. Alopecia areata is a common dermatologic disease affecting approximately 2.5 million individuals in the U.S., which presents with round, patchy hair loss on the scalp and has been postulated to have an underlying autoimmune component to its pathomechanism. Rook and Dawber,

10 1991, *Diseases of the Hair and Scalp* (Blackwell Press, Oxford, UK, ed. 2,) pp. 136-166; Bergfeld, 1995, *Am. J. Med.* 98:95S-98S. Alopecia areata can progress to involve hair loss of the entire scalp, and is referred to as alopecia totalis. Alopecia universalis is the term for the most

15 extreme example of disease progression, resulting in complete absence of scalp and body hair. *Id.* It is clear that alopecia areata is a "complex" genetic disorder resulting from more than one gene. In addition to these putative "autoimmune" forms of alopecia, a simple, recessively

20 inherited form also exists, known as "congenital alopecia universalis or "congenital atrichia". The precise etiology of this disorder is unknown, and prior to the present invention, no autoantigen or causative gene has been identified. Muller et al., 1980, *Br. J. Dermatol.* 102:609.

Summary of the Invention

5 The present invention provides an isolated nucleic acid which encodes a wildtype human hairless protein. The present invention further provides an isolated nucleic acid which encodes mutant human hairless proteins. The present invention further provides an isolated wildtype human hairless protein and also provides an isolated mutant human hairless protein.

10 In addition, the present invention provides a method of isolating a nucleic acid encoding a wildtype human hairless-related protein in a sample containing nucleic acid comprising (a) contacting the nucleic acid in the sample with  
15 the nucleic acid probe provided herein, under conditions permissive to the formation of a hybridization complex between the nucleic acid probe and the nucleic acid; (b) isolating the complex formed; and (c) separating the nucleic acid probe and the nucleic acid, thereby isolating the  
20 nucleic acid encoding a wildtype human hairless protein in the sample.

Further, the present invention provides a method for identifying a compound which is capable of enhancing or  
25 inhibiting expression of a human hairless protein comprising: (a) contacting a cell which expresses the human hairless protein in a cell and the compound; (b) determining the level of expression of the human hairless protein in the cell; and  
30 (c) comparing the level of expression of the human hairless protein determined in step (b) with the level determined in the absence of the compound, thereby identifying a compound capable of inhibiting or enhancing expression of the human hairless protein.

35 The present invention also provides a method for identifying a binding compound which is capable of forming a complex with a human hairless protein comprising: (a) contacting the human hairless protein and the compound; and (b) determining the

formation of a complex between the human hairless protein and the compound, thereby identifying a binding compound which is capable of forming a complex with a human hairless protein.

5

The present invention additionally provides a method for identifying an inhibitory compound which is capable of interfering the capacity of a human hairless protein to form a complex with the binding compound comprising: (a) contacting the complex and the compound; (b) measuring the level of the complex; and (c) comparing the level of complex in the presence of the compound with the amount of the complex in the absence of the compound, a reduction in level of complex thereby identifying an inhibitory compound which is capable interfering the capacity of a human hairless protein to form a complex with the binding compound.

10

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Also, the present invention provides a transgenic non-human animal comprising a nucleic acid encoding a human hairless protein (wildtype or mutant).

25

30

Further still, the present invention provides a method for identifying whether a compound is capable of ameliorating a human hairless condition in an animal comprising: (a) administering the compound to a transgenic animal wherein the animal exhibits a human hairless condition; (b) determining the level of expression of the protein of human hairless protein (wildtype or mutant); and (c) comparing the level expression of the human hairless protein (wildtype or mutant) determined in step (b) with the level of expression determined in the animal in the absence of the compound so as to identify whether the compound is capable of ameliorating the human hairless condition in the animal.

35

The present invention also further provides a transgenic non-human knockout animal whose cells do not express a gene encoding the human hairless protein (wildtype or mutant).

This invention further provides a method for identifying a compound capable of restoring normal phenotype to the animal provided herein comprising (a) administering the compound to the animal, wherein the animal exhibits a human hairless condition; (b) comparing the exhibition of the condition in the animal in the presence of the compound with the exhibition of the condition in the animal in the absence of the compound so as to identify whether the compound is capable of restoring normal phenotype to the animal.

10

This invention also provides a pharmaceutical composition which comprises a compound identified by the methods disclosed herein and a pharmaceutically acceptable carrier.

15 The present invention additionally provides a method for treating a human hairless condition in a subject comprising administering to the subject an amount of the pharmaceutical composition disclosed herein, effective to treat the human hairless condition in the subject.

20

The present invention also provides an antibody which binds specifically to the human hairless protein (wildtype or mutant) or portion thereof. The present invention provides a cell producing the antibody provided herein. The present invention further provides a method of identifying the human hairless protein (wildtype or mutant) in a sample comprising (a) contacting the sample with the antibody provided herein under conditions permissive to the formation of a complex between the antibody and the protein; (b) determining the amount of complex formed; and (c) comparing the amount of complex formed with the amount of complex formed in the absence of the sample, the presence of an increased amount of complex formed in the presence of the sample indicating identification of the protein in the sample.

30

35 Finally, the present invention provides a method of inhibiting hair growth, comprising administering to the subject an amount of the pharmaceutical composition provided

herein, effective to inhibit hair growth in the subject.

Brief Description of the Figures for the First Series of Experiments

**Figure 1**

5        The pedigree of the Alopecia universalis (AU) family over six generations. Black circles and squares represent affected females and males, respectively, and figures with a black dot at the center represent heterozygous carriers. The grey shaded box beneath the  
10        pedigree characters indicates the haplotype on chromosome 8p that cosegregates with the disease. The order of the markers is indicated in the lower right corner.

15        **Figures 2A-2C**

         Clinical presentation of the congenital alopecia universalis phenotype (A) Note the complete absence of hair over the entire scalp of an affected individual (V-11 in Figure 1). (B) The eyebrows, eyelashes and  
20        facial hair are completely missing. (C) Histopathology of a scalp biopsy from the same individual revealed a markedly reduced number of hair follicles and those present were found to be dilated and without hairs (lower left). Note the absence of an inflammatory  
25        infiltrate. (D) Clinical presentation of a child with congenital alopecia and T-cell immunodeficiency. Note the complete absence of frontal scalp hair, eyebrows and eyelashes in this five year old young girl (left panel). Scalp hair is completely missing on the entire  
30        head (right panel).

**Figure 3A-3B**

         (A) The lod score calculations for the linkage of AU to chromosome 8p12 markers for the congenital alopecia  
35        universalis family. (B) Comparison of the linkage interval defined in the congenital alopecia universalis

family with the location of the human *hairless* (*hr*) gene (right) established by radiation hybrid mapping. By linkage analysis, the locus of the gene in the AU family was predicted to lie within the 6-cM interval defined by the markers D8S258 and D8S1739 (left). By radiation hybrid mapping, the *hairless* gene was predicted to lie within the 19-cM interval between the markers D8D280 and D8S278 (right), thus making it a strong candidate gene in the congenital alopecia universalis family.

#### Figures 4A-4C

(A). Sequence comparison of human (H) (Seq.ID.No.:4), mouse (M) (Seq.ID.No.:5) and rat (R) *hairless* (Seq.ID.No.:3). Areas shaded in black represent regions of complete homology, those shaded in grey, represent conservative amino acid substitutions, and areas in white represent nonconservative substitutions. The homology of human *hairless* compared with mouse and rat was 84% and 83% respectively. The conserved six-cysteine motif is indicated by asterisks beneath the sequence. The human sequence represents Seq.ID.No.:3.

(B) Northern blot analysis of human *hairless* (*hr*) in poly(A)+ mRNA from eight different tissues, revealing a ~5 kb message (arrow). Lanes 1 to 8 show heart, brain, placenta, lung, liver skeletal muscle, kidney, and pancreas, respectively. Substantial expression is noted only in the brain (lane 2), with trace expression elsewhere (lanes 1 and 3 to 8).

(C) Northern blot analysis of human *hairless* in poly (A)+ mRNA from culture fibroblasts derived from hair-bearing skin reveals the same size *hairless* message (arrow).

Mutation analysis of exon 15 of the human *hairless* gene in the congenital alopecia universalis family. (A) The wild-type sequence contains a homozygous A (arrow) at the first base of a threonine codon (ACA). (B) Sequence analysis of heterozygous carriers in the congenital alopecia universalis family reveals the presence of a G as well as the wild-type A at this position (arrow). (C) Sequencing of all affected individuals in the congenital alopecia universalis family reveals a homozygous mutant G at this position (arrow), resulting in the substitution of threonine by alanine (GCA).

#### Figures 6

The nucleic acid sequence of nucleic acid encoding human *hairless* wildtype protein (Seq.ID.No.:1 and Seq.ID.No.:2).

#### Brief Description of the Figures for the Second Series of Experiments

#### Figure 7A-7B

(A) Pedigree of the family shown with disease associated haplotypes. Filled circles and diamonds indicate affected females and individuals of unknown gender, respectively, and half-filled circles and squares represent heterozygous carriers of the mutation. Double are indicative of a consanguineous union. Haplotypes are listed vertically beneath each character from whom DNA was available. The disease-associated haplotype is framed in a grey box beneath each figure, and the order of the markers with respect to the *whn* gene is given in the box at the lower right. Mutation status with respect to the *whn* gene was scored as 1=wild-type allele; 2-mutant R255X allele.



Recombination events in individual IV-6 are indicated by arrows on either side of the haplotypes. (B) The lod score calculations for the linkage to the *whn* gene mutation.

5

**Figure 8A-8D**

(A) Sequence analysis of a nonsense mutation in exon 5 of the *whn* gene. The upper panel reveals the homozygous wild-type *whn* sequence in exon 5, from an unrelated, unaffected control individual. The middle panel contains DNA sequence from a heterozygous carrier of the mutation R255X. Note the double T+C peak directly beneath the arrow. The lower panel represents the homozygous mutant R255X sequence. Note the presence of the mutant T only beneath the arrow, leading to a C-to-T transition and a substitution of an arginine residue by a nonsense mutation CGA-to-TGA, possibly due to spontaneous demethylation at the CpG dinucleotide. (B) Confirmation of the mutation by restriction enzyme digestion. The mutation introduced a new restriction site for *Bsr*I, and after digestion of the 184 bp PCR product containing exon 5, the product generated from the mutant allele should cleave into two bands of 120 and 64 bp in size. The clinically unaffected parents and brother revealed three bands of 184 bp, 120 bp, and 64 bp (lanes 1, 2 and 6, upper panel), indicating that they were heterozygous carriers of the mutation R255X. Both patients revealed only the two digested bands of 120 bp and 64 bp in size (lanes 3 and 4), consistent with the presence of the mutation in the homozygous state. (C) Evidence for long-term engraftment of the BMT. Gender determination of the family members revealed a genotypically XX pattern of an undigested 300 bp band in the mother (lane 1) and affected patients (lanes 3

and 4), and a genotypically XY pattern consisting of the 300 bp band and two additional bands of 216 and 84 bp, indicative of the Y chromosome in the brother (lane 2) and the father (lane 6). Lane 5 contains peripheral blood leukocyte from the patient after BMT, demonstrating an XY genotype and the presence of the normal *whn* allele, providing evidence for fraternal chimerism and persistence of the graft. (D) Sequence analysis of the hairless gene (Top) the wildtype sequence of exon 3 (Middle) Sequence analysis of a heterozygous carrier (Bottom) The 22-bp deletion in the homozygous state in an affected individual. The arrow and bar above the wildtype sequence in the top panel represent the sequence that is deleted in the homozygous state in the patient in the bottom panel.

#### Figure 9A-9D

Expression of *whn* in different human tissues. (A) Hybridization of the dot blot with a probe specific for human *whn* revealed a strong signal in only three tissue sources: Adult thymus (dot E5), fetal thymus (dot G6) and human genomic control DNA (dot H8). (B) Hybridization of the human immune system northern blot revealed expression only in lane 3 containing thymus RNA. Lane 1 contains spleen mRNA; lane 2, lymph node; lane 4, peripheral blood leukocyte; lane 5, bone marrow; and lane 6, fetal liver. (C) Hybridization of the human multiple tissue northern blot revealed expression only in lane 2 containing thymus RNA. Lane 1 contains spleen mRNA; lane 3, prostate; lane 4, testis; lane 5, ovary; lane 6, small intestine; lane 7, colon without mucosa; and lane 8, peripheral blood leukocyte. (D) Northern analysis of skin fibroblasts (lane 1) and epidermal keratinocytes (lane 2) reveals strong expression of *whn* in keratinocytes and

negligible expression in fibroblasts (upper panel), despite marked overloading of the fibroblast mRNA in lane 1 as ascertained by GAPDH signal as internal control (lower panel). There is a faint, minor transcript present in the keratinocyte RNA that is not observed in thymus RNA.

#### Figure 10A-10D

*Whn* mRNA expression in normal human scalp skin. *In situ* hybridization with a digoxigenin-labeled *whn* complementary RNA probe in sections of paraffin embedded skin samples. (A) In interfollicular epidermis, *whn* mRNA is concentrated in the basal keratinocytes and the suprabasal cell layers of the spinous compartment. It declines gradually with keratinocyte differentiation and is prominently reduced or absent in upper spinous cells and in granular cell layer. (B) The sweat gland (SW) epithelium and proliferating cells of the sebaceous gland (SG) epithelium are always *whn* mRNA positive. In the distal portion of the anagen hair follicle epithelium, *whn* mRNA expression is localized to the basal cell layer of the outer root sheath (ORS) (arrow). (C) The innermost cell layer of the ORS is always highly *whn* mRNA positive (arrows). (D) In the proximal portion of the hair bulb, *whn* mRNA is localized to the differentiating cells of the hair matrix (HM) and the innermost ORS cell layer (arrowhead), while the dermal papilla (DP) fibroblasts and inner root sheath (arrow) remain *whn* mRNA negative.

#### Figure 11

Summary of existing mutations in the human hairless gene, consisting of missense, nonsense and deletion mutations. Ahmad, 1998, *Science* 279:720-724; Ahmad,

1998, *Am. J. Hum. Genet.* 63:984-991; Ahmad, 1998, *Human. Genet.* 63:984-991.

**Figure 12A-12B**

5       Antibodies which bind specifically to the human  
hairless protein. (A) Total protein lysates of 293T  
cells transiently transfected with either control  
plasmid or plasmid containing the Hr cDNA FLAG-tagged  
at the amino-terminus, were used in immuno  
10       precipitation experiments using either anti-FLAG  
antibodies or an Hr immune serum. Immuno precipitates  
were separated by SDS-PAGE and immunoblot analysis was  
done using anti-FLAG antibodies. Both the anti-FLAG  
and Hr immune serum are able to specifically immuno  
15       precipitate Hr proteins. (B) Total protein lysates of  
293T cells transiently transfected with either control  
plasmid or plasmid containing the Hr cDNA, were  
separated by SDS-PAGE. Immunoblot analysis was done  
using 4 serial dilutions of either pre-immune serum or  
20       an immune serum generated against the Hr protein. The  
Hr immune serum specifically detects a 122kD protein,  
which corresponds to the predicted molecular weight of  
the Hr protein.

25

Detailed Description of the Invention

The present invention provides an isolated nucleic acid which encodes a wildtype human hairless protein. The present invention further provides an isolated nucleic acid which encodes a mutant human hairless protein. The present invention further provides an isolated wildtype human hairless protein and also provides isolated mutant human hairless proteins.

In an embodiment of this invention the nucleic acid is DNA. In another embodiment of this invention, the nucleic acid is RNA. In still another embodiment the nucleic acid is cDNA. In yet another embodiment, the nucleic acid is genomic DNA. In an embodiment of the invention the nucleic acid comprises a nucleic acid having a sequence substantially the same as the sequence designated SEQ. ID. No.: 1. In still another embodiment, the nucleic acid comprises a nucleic acid (Seq.ID.No.:2) having the sequence of SEQ. ID. No.: 1 except a G to A transition occurs at the first base of a threonine (T) residue at position 1022 (ACA) converting the threonine residue to an alanine (A) residue as indicated for the human sequence (H) in Figure 1. In another embodiment, the nucleic acid comprises a nucleic acid having a sequence substantially the same as the sequence designated SEQ. ID. No.: 1 and wherein a nucleotide transition occurs at a threonine (T) residue at position 1022 (ACA) converting the threonine residue to an alanine (A) residue as indicated for the human sequence (H) in Figure 1. In still another embodiment, the nucleic acid comprises a nucleic acid having a sequence substantially the same as the sequence designated SEQ. ID. No.: 1 and wherein a nucleotide transition occurs at a threonine (T) residue at position 1022 (ACA) converting the threonine to a different amino acid residue. In a final embodiment, the nucleic acid comprises a nucleic acid having a sequence substantially the same as the sequence designated SEQ. ID. No. 1 wherein the nucleotide transition occurs at a residue for hairlessness converting the amino acid residue

in the region to a different amino acid.

5 An embodiment of this invention is a vector comprising the nucleic acid molecule. In an embodiment of this invention, the vector is a virus, cosmid, yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), bacteriophage or a plasmid. An embodiment of this invention is a host vector system for the production of a human hairless protein which comprises the vector in a suitable host. In an  
10 embodiment of this invention, the suitable host is a bacterial cell or a eukaryotic cell. In an embodiment of this invention, the suitable host is a mammalian cell, yeast or insect cell.

15 Another embodiment of the present invention is a nucleic acid probe comprising a nucleic acid of at least 11 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within the nucleic acid encoding wildtype or mutant human hairless protein. In an embodiment of this  
20 invention, the nucleic acid probe is DNA or RNA. In another embodiment of this invention, the nucleic acid is in the antisense orientation to the coding strand of the nucleic acid encoding the mutant or wildtype human hairless protein.

25 Another embodiment of the present invention is the isolated human hairless wildtype protein having substantially the same amino acid sequence as the human amino acid sequence shown in Figure 4 and designated herein as SEQ.ID.NO.: 3.

30 Yet another embodiment of the present invention is the isolated human hairless mutant protein having substantially the same amino acid sequence as the human amino acid sequence shown in Figure 4 except the threonine (T) at position 1022 is replaced by alanine (A) and is designated herein as  
35 SEQ.ID.NO.: 4. In another embodiment of this invention, the protein having substantially the same amino acid sequence as the human amino acid sequence (H) shown in Figure 4 (SEQ.ID.NO.: 3). Yet another embodiment, is the protein

having substantially the same amino acid sequence as the human amino acid sequence (H) shown in Figure 4 (SEQUENCE ID NO.: 3) except the threonine (T) at position 1022 is replaced by alanine (A) and is designated herein as SEQ.ID.NO.: 4.

5 Still another embodiment is the protein having substantially the same amino acid sequence as the human amino acid sequence (H) shown in Figure 4 (SEQUENCE ID NO.: 3) except the threonine (T) at position 1022 is replaced by an amino acid other than alanine.

10

In addition, the present invention provides a method of isolating a nucleic acid encoding a wildtype human hairless-related protein in a sample containing nucleic acid comprising (a) contacting the nucleic acid in the sample with  
15 the nucleic acid probe provided herein, under conditions permissive to the formation of a hybridization complex between the nucleic acid probe and the nucleic acid; (b) isolating the complex formed; and (c) separating the nucleic acid probe and the nucleic acid from the isolated complex  
20 resulting from step (b), thereby isolating the nucleic acid encoding a wildtype human hairless-related protein in the sample.

In another embodiment, the isolated wildtype human whn  
25 protein has a homozygous arginine to a premature termination codon transition (C-to-T) at nucleotide position 792 leading to a mutation at amino acid position 255 of the protein.

An embodiment of this invention is further comprising (a)  
30 amplifying the nucleic acid in the sample under conditions permissive to polymerase chain reaction; and (b) detecting the presence of a polymerase chain reaction product, the presence of polymerase chain reaction product identifying the presence of a nucleic acid encoding a human hairless-related  
35 protein in the sample. An embodiment of this invention is the nucleic acid isolated by this method. Yet another embodiment is the detection of the polymerase chain reaction product which comprises contacting the nucleic acid molecule

from the sample with the nucleic acid probe described herein, wherein the nucleic acid probe is labeled with a detectable marker. Still another embodiment of this invention is wherein the detectable marker is a radiolabeled molecule, a  
5 fluorescent molecule, an enzyme, a ligand, or a magnetic bead.

Further, the present invention provides a method for identifying a compound which is capable of enhancing or  
10 inhibiting expression of a human hairless protein comprising: (a) contacting a cell which expresses the human hairless protein in a cell and the compound; (b) determining the level of expression of the human hairless protein in the cell; and  
15 (c) comparing the level of expression of the human hairless protein determined in step (b) with the level determined in the absence of the compound, thereby identifying a compound capable of inhibiting or enhancing expression of the human hairless protein.

20 In embodiment of this invention, step (a) comprises contacting a nucleic acid which expresses the human hairless protein in a cell-free expression system and the compound. An embodiment of this invention is a compound, not previously known, identified by this method. According to an embodiment  
25 of this invention, the cell is a dermal papilla cell, an epithelial cell, a follicle cell, a hair matrix cell, a hair bulb cell, a keratinocyte, an epidermal keratinocyte, a fibroblast, a cuticle cell, a medullary cell, a cortical cell or a thymic cell. According to an embodiment of this  
30 invention, the compound is a peptide, a peptidomimetic, a nucleic acid, a polymer, or a small molecule. In one embodiment of this invention, the compound is bound to a solid support.

35 The present invention also provides a method for identifying a binding compound which is capable of forming a complex with a human hairless protein comprising: (a) contacting the human hairless protein and the compound; and (b) determining the



formation of a complex between the human hairless protein and the compound, thereby identifying a binding compound which is capable of forming a complex with a human hairless protein.

5

An embodiment of this invention is a compound, not previously known, identified by this method, capable of forming a complex with a human hairless protein.

10 The present invention additionally provides a method for identifying an inhibitory compound which is capable of interfering the capacity of a human hairless protein to form a complex with the binding compound comprising: (a) contacting the complex and the compound; (b) measuring the  
15 level of the complex; and (c) comparing the level of complex in the presence of the compound with the amount of the complex in the absence of the compound, a reduction in level of complex thereby identifying an inhibitory compound which is capable interfering the capacity of a human hairless  
20 protein to form a complex with the binding compound.

An embodiment of this invention is a compound, not previously known, identified by the method described, capable of  
25 interfering with the capacity of a human hairless protein to form a complex with the identified binding compound.

Also, the present invention provides a transgenic non-human animal comprising a nucleic acid encoding wildtype or mutant human hairless protein. An embodiment of this invention is  
30 a transgenic non-human animal whose somatic and germ cells contain and express a gene encoding the human hairless protein (wildtype or mutant) or the *whn* protein, the gene having been introduced into the animal or an ancestor of the animal at an embryonic stage and wherein the gene may be  
35 operably linked to an inducible promoter element. In one embodiment of this invention, the animal is a mouse.

Further still, the present invention provides a method for

identifying whether a compound is capable of ameliorating a human hairless condition in an animal comprising: (a) administering the compound to a transgenic animal wherein the animal exhibits a human hairless condition; (b) determining  
5 the level of expression of the protein of human hairless protein (wildtype or mutant); and (c) comparing the level expression of the human hairless protein (wildtype or mutant) determined in step (b) with the level of expression  
10 as to identify whether the compound is capable of ameliorating the human hairless condition in the animal.

An embodiment of this invention is a compound, not previously known, identified by this method, capable of ameliorating a  
15 human hairless condition in an animal. In embodiment of this invention, the human hairless condition is Androgenetic Alopecia (male pattern baldness), Alopecia Areata , Alopecia Totalis, Alopecia Universalis, Congenital Alopecia Universalis or Congenital Alopecia and Severe- T-Cell  
20 Immunodeficiency.

The present invention also further provides a transgenic non-human knockout animal whose cells do not express a gene encoding a mutant or wildtype human hairless protein. An  
25 embodiment of this invention is a transgenic non-human knockout animal whose somatic and germ cells do not express a gene encoding the human hairless protein (wildtype or mutant), the gene(s) having been deleted or incapacitated in the animal or an ancestor of the animal at an embryonic  
30 stage. In an embodiment of this invention, the animal is a mouse.

This invention further provides a method for identifying a compound capable of restoring normal phenotype to the animal  
35 provided herein comprising (a) administering the compound to the animal, wherein the animal exhibits a human hairless condition; (b) comparing the exhibition of the condition in the animal in the presence of the compound with the

exhibition of the condition in the animal in the absence of the compound so as to identify whether the compound is capable of restoring normal phenotype to the animal. An embodiment of this invention is a compound, not previously known, identified by this method capable of restoring normal phenotype to the animal. In an embodiment of this invention, the human hairless condition is Androgenetic Alopecia (male pattern baldness), Alopecia Areata, Alopecia Totalis, Alopecia Universalis, Congenital Alopecia Universalis or Congenital Alopecia and Severe T-Cell Immunodeficiency.

This invention also provides a pharmaceutical composition which comprises a compound identified by the methods disclosed herein and a pharmaceutically acceptable carrier. In an embodiment of this invention, the carrier is a diluent, an aerosol, a topical carrier, an aqueous solution, a nonaqueous solution or a solid carrier.

The present invention additionally provides a method for treating a human hairless condition in a subject comprising administering to the subject an amount of the pharmaceutical composition disclosed herein, effective to treat the human hairless condition in the subject. According to an embodiment of this invention, the human hairless condition is Androgenetic Alopecia (male pattern baldness), Alopecia Areata, Alopecia Totalis or Alopecia Universalis, Congenital Alopecia Universalis or Congenital Alopecia and Severe T-Cell Immunodeficiency.

The present invention also provides an antibody which binds specifically to the human hairless protein (wildtype or mutant) or portion thereof. The present invention provides a cell producing the antibody provided herein. The present invention further provides a method of identifying the human hairless protein (wildtype or mutant) in a sample comprising (a) contacting the sample with the antibody provided herein under conditions permissive to the formation of a complex between the antibody and the protein; (b) determining the

amount of complex formed; and (c) comparing the amount of complex formed with the amount of complex formed in the absence of the sample, the presence of an increased amount of complex formed in the presence of the sample indicating identification of the protein in the sample. According to one embodiment of this invention, the antibody is human or mouse. According to an embodiment of this invention, the antibody is a monoclonal antibody. An embodiment of this invention also provides a cell producing the antibody which binds specifically to a mutant or wildtype human hairless protein. An embodiment of this invention further provides a method of identifying a mutant or wildtype human hairless protein comprising: (a) contacting the sample with the antibody under conditions permissive to the formation of a complex between the antibody and the protein; (b) determining the amount of complex formed; and (c) comparing the amount of complex formed with the amount of complex formed in the absence of the sample, the presence of an increased amount of complex formed in the presence of the sample indicating identification of the protein in the sample.

Finally, the present invention provides a method of inhibiting hair growth, comprising administering to the subject an amount of the pharmaceutical composition provided herein, effective to inhibit hair growth in the subject.

As used herein, the term "human hairless protein" shall mean polypeptides encoded by the human polypeptide sequence marked (H) set forth in Figure 4 and designated herein as Seq.ID.No.:3 and any polypeptide which possesses substantial amino acid homology with said polypeptides.

As used herein, the term "human hairless polynucleotide" shall mean: (1) polynucleotides encoded by the polynucleotide sequence set forth in Figure 6 and designated herein as Seq.ID.No.:1, (2) any polynucleotide sequence which encodes for a human hairless protein or (3) any polynucleotide

sequence which hybridizes to the polynucleotide sequences of (1) and (2), above, under stringent hybridization conditions.

As used herein, "stringent hybridization conditions" are those hybridizing conditions that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015M sodium citrate/0.1% SDS at 50°C; (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/ 50mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate) 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

As used herein, "substantial amino acid homology" shall mean molecules having a sequence homology of approximately 85% or more, preferably greater than or equal to 90% and more preferably greater than or equal to 95%.

The present invention relates to the human polypeptide and polynucleotide molecules and sequences which correspond to a factor implicated in the development of the hair follicle and in the hair cycle. This factor, designated the human hairless protein, and specifically, the expression of mutated forms of this factor, are related to abnormal hair growth, including alopecias.

The present invention is further directed to methods for manipulating the expression of the human hairless protein to interrupt the hair cycle, either by manipulating hair follicle development or one of the stages of the hair growth cycle. Such methods may be useful to inhibit hair growth. In one embodiment of the invention, methods and compositions which rely upon the manipulation of the signal peptide which

corresponds to the human hairless protein. In the preferred methods and compositions, the compositions are applied topically to the area in which hair growth is sought to be regulated.

5

The practice of the present invention may include expression of biologically active human hairless protein. In order to express a biologically active human hairless, the nucleotide sequence coding for the protein, or a functional equivalent may be inserted into an appropriate expression vector, i.e.,  
10 a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

15

More specifically, methods which are well known to those skilled in the art can be used to construct expression vectors containing the human hairless sequence and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques,  
20 synthetic techniques and in vivo recombination/genetic recombination. See e.g., the techniques described in Maniatis et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing  
25 Associates and Wiley Interscience, N.Y.

30

A variety of host-expression vector systems may be utilized to express the human hairless coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA  
or cosmid DNA expression vectors containing the human hairless coding sequence; yeast transformed with recombinant yeast expression vectors containing the human hairless coding  
35 expression vectors (e.g., baculovirus) containing the Human hairless coding sequence; plant cell systems infected with

recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the Human hairless coding sequence; or  
5 animal cell systems infected with recombinant virus expression vectors (e.g., adenovirus, vaccinia virus, human tumor cells (including HT-1080)) including cell lines engineered to contain multiple copies of the Human hairless DNA either stably amplified (CHO/dhfr) or unstably amplified  
10 in double-minute chromosomes (e.g., murine cell lines).

The expression elements of these systems vary in their strength and specificities. Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and  
15 inducible promoters, may be used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage (plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when  
20 cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter may be used; when cloning in plant cell systems, promoters derived from the genome of plant cells (e.g., heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll  
25 a/b binding protein) or from plant viruses (e.g., the 35S RNA promoter of CaMV; the coat protein promoter of TMV) may be used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g.,  
30 the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used; when generating cell lines that contain multiple copies of the Human hairless DNA SV40-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

35

In bacterial systems, a number of expression vectors may be

advantageously selected depending upon the use intended for the expressed Human hairless. For example, when large quantities of Human hairless for screening purposes, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include but are not limited to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the Human hairless coding sequence may be ligated into the vector in frame with the lac Z coding region so that a hybrid AS-lac Z protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety.

In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant et al., 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 1987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; Bitter, 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and The Molecular Biology of the Yeast Saccharomyces, 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II.



In cases where plant expression vectors are used, the expression of the Human hairless coding sequence may be driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson et al., 1984, *Nature* 310:511-514), or the coat protein promoter of TMV (Takamatsu et al., 1987, *EMBO J.* 6:307-311) may be used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al., 1984, *EMBO J.* 3:1671-1680; Broglie et al., 1984, *Science* 224:838-843); or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley et al., 1986, *Mol. Cell. Biol.* 6:559-565) may be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, microinjection, electroporation, etc. For reviews of such techniques see, for example, Weissbach & Weissbach, 1988, *Methods for Plant Molecular Biology*, Academic Press, NY, Section VIII, pp. 421-463; and Grierson & Corey, 1988, *Plant Molecular Biology*, 2d Ed., Blackie, London, Ch. 7-9.

An alternative expression system which could be used to express Human hairless is an insect system. In one such system, baculovirus may be used as a vector to express foreign genes. The virus then grows in the insect cells. The Human hairless coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of a Baculovirus promoter. These recombinant viruses are then used to infect insect cells in which the inserted gene is expressed. (E.g., see Smith et al., 1983, *J. Virol.* 46:584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the Human hairless coding sequence may be ligated to an adenovirus

transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing Human hairless in infected hosts. See e.g., Logan & Shenk, 1984, Proc. Natl. Acad. Sci. (USA) 81:3655-3659. Alternatively, the vaccinia 7.5K promoter may be used. See, e.g., Mackett et al., 1982, Proc. Natl. Acad. Sci. (USA) 79:7415-7419; Mackett et al., 1984, J. Virol. 49:857-864; Panicali et al., 1982, Proc. Natl. Acad. Sci. 79:4927-4931.

In another embodiment, the Human hairless sequence is expressed in human tumor cells, such as HT-1080, which have been stably transfected with calcium phosphate precipitation and a neomycin resistance gene.

Specific initiation signals may also be required for efficient translation of inserted Human hairless coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where the entire Human hairless gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the Human hairless coding sequence is inserted, exogenous translational control signals, including the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the Human hairless coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. See e.g.,

Bitter et al., 1987, Methods in Enzymol. 153:516-544.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cells lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, WI38, HT-1080 etc.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express Human hairless may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with Human hairless DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk<sup>-</sup>, hgprt or ap<sup>r</sup>t cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Proc. Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hyg<sup>r</sup>, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147) genes. Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, 1988, Proc. Natl. Acad. Sci. USA 85:8047), and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.).

In the practice of the present invention, a transgenic animal may be generated. One means available for generating a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (Hogan B. et al. Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)). DNA or cDNA encoding a vertebrate hairless protein is purified from a vector by

methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

In the practice of any of the methods of the invention or preparation of any of the pharmaceutical compositions an "therapeutically effective amount" is an amount which is capable of inhibiting hairlessness or T-cell deficiency. Accordingly, the effective amount will vary with the subject being treated, as well as the condition to be treated. For the purposes of this invention, the methods of administration are to include, but are not limited to, administration cutaneously, subcutaneously, intravenously, parenterally, orally, topically, or by aerosol.

As used herein, the term "suitable pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutically accepted carriers, such as phosphate buffered saline solution, water, emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules. An example of an acceptable triglyceride emulsion useful in intravenous and intraperitoneal administration of the

compounds is the triglyceride emulsion commercially known as Intralipid®.

Typically such carriers contain excipients such as starch,  
5 milk, sugar, certain types of clay, gelatin, stearic acid, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients.

10 This invention also provides for pharmaceutical compositions capable of inhibiting neurotoxicity together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions are liquids or lyophilized or otherwise dried formulations and include  
15 diluents of various buffer content (e.g., Tris-HCl., acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), solubilizing agents (e.g., glycerol, polyethylene glycerol),  
20 anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the compound, complexation with metal  
25 ions, or incorporation of the compound into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, micro emulsions, micelles, unilamellar or multi lamellar vesicles, erythrocyte ghosts, or spheroplasts. Such  
30 compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance of the compound or composition.

Controlled or sustained release compositions include  
35 formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g., poloxamers or poloxamines) and the compound coupled to antibodies directed

against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral.

When administered, compounds are often cleared rapidly from the circulation and may therefore elicit relatively short-lived pharmacological activity. Consequently, frequent injections of relatively large doses of bioactive compounds may be required to sustain therapeutic efficacy. Compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline are known to exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds (Abuchowski et al., 1981; Newmark et al., 1982; and Katre et al., 1987). Such modifications may also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result, the desired *in vivo* biological activity may be achieved by the administration of such polymer-compound adducts less frequently or in lower doses than with the unmodified compound.

Attachment of polyethylene glycol (PEG) to compounds is particularly useful because PEG has very low toxicity in mammals (Carpenter et al., 1971). For example, a PEG adduct of adenosine deaminase was approved in the United States for use in humans for the treatment of severe combined immunodeficiency syndrome. A second advantage afforded by the conjugation of PEG is that of effectively reducing the immunogenicity and antigenicity of heterologous compounds. For example, a PEG adduct of a human protein might be useful

for the treatment of disease in other mammalian species without the risk of triggering a severe immune response. The carrier includes a microencapsulation device so as to reduce or prevent an host immune response against the compound or  
5 against cells which may produce the compound. The compound of the present invention may also be delivered microencapsulated in a membrane, such as a liposome.

10 Polymers such as PEG may be conveniently attached to one or more reactive amino acid residues in a protein such as the alpha-amino group of the amino terminal amino acid, the epsilon amino groups of lysine side chains, the sulfhydryl groups of cysteine side chains, the carboxyl groups of aspartyl and glutamyl side chains, the alpha-carboxyl group  
15 of the carboxy-terminal amino acid, tyrosine side chains, or to activated derivatives of glycosyl chains attached to certain asparagine, serine or threonine residues.

20 Numerous activated forms of PEG suitable for direct reaction with proteins have been described. Useful PEG reagents for reaction with protein amino groups include active esters of carboxylic acid or carbonate derivatives, particularly those in which the leaving groups are N-hydroxysuccinimide, p-nitrophenol, imidazole or 1-hydroxy-2-nitrobenzene-4-sulfonate.  
25 PEG derivatives containing maleimido or haloacetyl groups are useful reagents for the modification of protein free sulfhydryl groups. Likewise, PEG reagents containing amino hydrazine or hydrazide groups are useful for reaction with aldehydes generated by periodate oxidation of  
30 carbohydrate groups in proteins.

This invention is illustrated by examples set forth in the Experimental Details section which follows. This section is provided to aid in an understanding of the invention but is  
35 not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.



## EXPERIMENTAL DETAILS

### First Series of Experiments

Example 1: Identification of the human hairless gene. In an effort to understand the molecular basis of an inherited form of congenital alopecia universalis, a Pakistani kindred with congenital alopecia universalis segregating as a single abnormality without associated ectodermal defects was identified and studied. This kindred was comprised of 4 affected males and 7 affected females (Figure 1). At birth, the hair usually appears normal on the scalp, but never regrows after ritual shaving usually performed a week after birth. Skin biopsy from the scalp of an affected person revealed very few hair follicles, dilated, and without hairs. Affected persons are born completely devoid of eyebrows and eyelashes, and never develop axillary and pubic hair. The pedigree is strongly suggestive of autosomal recessive inheritance, and various consanguineous loops account for all affected persons being homozygous for the abnormal allele.

Locus determination. To identify the alopecia locus segregating in this family, a genome wide search for linkage was initiated using the homozygosity mapping approach. Sheffield, et al., 1995, *Curr. Opin Genet. Devel.* 5:335. During the initial screening, DNA samples from four affected individuals (IV-22, V-2, V-11, and VI-2 in Figure 4) were genotyped using 386 highly polymorphic microsatellite markers spaced at 10 cM intervals (Research Genetics, Inc.). More specifically, blood samples were collected from 36 members of the congenital alopecia universalis family, according to local informed consent procedures. DNA was isolated according to standard techniques. J. Sambrook, E.G. Fritsch, T. Maniatis, *Molecular Cloning, A Laboratory Manual*, (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989). Florescent automated genotyping for the genome-wide linkage search was carried out using 386 markers covering the

genome at approximately 10 cM intervals. In the course of this screen, 13 genomic regions were found to be homozygous for three to four affected individuals, each of these were tested further in 32 additional family members, and twelve  
5 of these were excluded. One marker, D8S136 on chromosome 8p12, was found to be homozygous in all 7 affected individuals. Further analysis with markers from this region resulted in the identification of homozygosity in all affected individuals for the markers D8S1786 and D8S298.  
10 Refined and more extensive screening of all regions showing homozygosity in affected and unaffected family members was carried out using primers obtained from Research Genetics, Inc., or in the Genome Data Base. Analysis of microsatellite markers consisted of end-labeling one primer using (<sup>33</sup>P dATP,  
15 a PCR reaction consisting of 7 minutes at 95°C, 1 minute, 55°C, 1 minute; 72°C, 1 minute; and electrophoresis in a 6% polyacrylamide gel (Sequa-gel, Action Scientific). Microsatellite markers were visualized by exposure of the gel to autoradiography, and genotypes were assigned by visual  
20 inspection. Allele patterns obtained with the markers D8S136 and D8S1786 indicated that these two markers are placed very close to each other on chromosome 8p12. Using the FASTLINK 3.0 package, a maximum two point LOD score of 6.19 at zero recombination gene was achieved with the marker D8S298, as  
25 set forth at Table 1:

TABLE 1

Lod Scores For Linkage To  
Chromosome 8p12 Markers

5	Locus	Recombination Factors						
		<u>0</u>	<u>0.01</u>	<u>0.05</u>	<u>0.1</u>	<u>0.2</u>	<u>0.3</u>	<u>0.4</u>
	D8S258	$\alpha$	2.57	2.85	2.63	1.87	1.01	0.32
	D8S298	6.19	6.04	5.45	4.70	3.16	1.65	0.47
10	D8S1786	4.92	4.83	4.43	3.92	2.87	1.79	0.76
	D8S1739	$\alpha$	1.74	2.64	2.61	1.92	1.00	0.22

15 Statistical calculations for linkage analysis were carried out using the computer program FASTLINK version 3.0P (Schaffer, 1996, *Hum Hered.* 46:226), which enables all inbreeding loops in the family to be retained, and the capability for two point analysis. Autosomal recessive with

20 complete penetrance was assumed using a disease allele frequency of 0.0001. LOD score was calculated using equal allele frequencies, and setting the frequency of the allele segregating with the disease at 0.9, to obtain results under the most stringent model. Multipoint analysis was not

25 possible due to the large number of inbreeding loops and complexity of the pedigree. The results indicate that the alopecia gene in this family mapped to chromosome 8p12. Recombinant haplotypes observed in individuals IV-2 and IV-7 placed the alopecia locus within a 6 cM interval between the

30 distal and proximal markers, D8S258 and D8S1739, respectively (FIG. 3) with no obvious candidate genes in this interval.

### Cloning of human hairless.

A hairless mouse has been previously reported, as set forth in Brooke, 1924, *J. Hered.* 15:173. This mouse was studied  
5 as a potential model for human alopecias. To this end, work was conducted to clone the human homolog of *hairless* using PCR primers based on the available murine cDNA sequence (GenBank accession #Z32675), as reported in Cachon-Gonzalez et al., 1994, *Proc. Natl. Acad. Sci. U.S.A.*, 91:7717. RT-PCR  
10 amplification of a segment corresponding to exons 13-18 in the murine sequence using human skin fibroblast mRNA as template was performed, and delineated the corresponding intron/exon borders in the human sequence. More specifically, for RT-PCR of human hairless cDNA sequences,  
15 total RNA was extracted from cultured skin fibroblasts from a control individual according to standard methods, as set forth in Sambrook, et al., 1989, *Molecular Cloning, A Laboratory Manual*, (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY ed. 2, 1989). Human hairless mRNAs were  
20 reverse transcribed with MMLV reverse transcriptase (Gibco, BRL), using an oligo-dT primer (Pharmacia). PCR was carried out using the following primers, constructed on the basis of the mouse *hairless* sequence (GenBank #z32675): sense:  
5'TGAGGGCTCTGCTCCTCTGC3' (Seq.ID.No.:7); antisense  
25 5'GCTGGCTCCCTGGTGGTAGA3' (Seq.ID.No.:6). PCR conditions were 95°C, 5 minutes, followed by 35 cycles of 95°C, 1 minute; 55°C, 1 minute; 72°C, 1 minute, using AmpliTaq Gold DNA polymerase (Perkin-Elmer). Following direct sequencing of the human cDNA, exon-based primers were designed and used  
30 to amplify genomic DNA sequences at both the 5' donor and 3' acceptor splice junctions. The human hairless sequence has been deposited in GenBank and accorded accession number AF039196.

35 The human and murine amino acid sequences in this region

were 89% homologous, and the exon sizes were well conserved. The murine hairless gene resides on mouse chromosome 14 (Cachon-Gonzalez, et al., 1994, *Proc. Natl. Acad. Sci. U.S.A.*, 91:7717), which shares synteny with the human chromosomes 8p and 14q, among others.

To determine the precise chromosomal localization of the human homolog of hairless, radiation hybrid mapping using the Genebridge 4 panel consisting 93 radiation induced human-hamster cell hybrids (Research Genetics, Inc.), placed the human homolog of the mouse hairless gene on chromosome 8p, between the polymorphic markers, D8S280 and D278, spanning a 19 cM region (FIG. 4). A portion of human hairless intron 13 was PCR amplified and used for radiation hybrid mapping using the G3 panel, by Research Genetics, Inc. Primers were as follows: sense: 5'TATGTCACCAAGGGCCAGCC3' (Seq.ID.No.: 8); and antisense: 5'TCAGGGTAGGGGGTCATGCC3' (Seq.ID.No.: 9). PCR conditions were 95°C, 5 minutes, followed by 35 cycles of 95 C, 1 minute; 55°C, 1 minute; 72 °C, 1 minute, using AmpliTaq Gold DNA polymerase (Perkin-Elmer). PCR primers specifically amplified human hairless, and did not cross-hybridize with the hamster DNA used in the radiation hybrid panel.

The amino acid and nucleic acid sequences identified by the methods set forth above are set forth in Figures 4 and 6 respectively.

#### Relationship of human hairless to Alopecia

Data provides that the 6 cM candidate region obtained for the congenital alopecia universalis gene by linkage analysis with flanking markers D8S258 and D8S1739, lies between markers D8S280 and D8S278 based on the Genome Data Base, the Center for Medical Genetics and the radiation hybrid map constructed by the Human Genome Mapping Center at Stanford

University (SHGC). Based on this genomic co-localization, its was contemplated that the human hairless gene became a major candidate gene responsible for congenital alopecia universalis in this family, and the search for a mutation was initiated.

The sequence contained within exon 15 revealed a homozygous A-to-G transition in all affected individuals, which was not present in the heterozygous state in obligate carriers within the family, and not found in unaffected family members. The G-to-A transition occurred at the first base of a threonine residue (ACA), leading to a missense mutation and converting it to an alanine residue (GCA). The mutation created a new cleavage site for the restriction endonuclease HgaI (GACGC), which was used to confirm the presence of the mutation in genomic DNA, in addition to direct sequencing.

PCR primers were designed to amplify individual exons from genomic DNA, and each exon was directly sequenced from affected individuals and compared to unaffected, unrelated controls. Primers for specific amplification of exon 15 were: sense: 5'AGTGCCAGGATTACAGGCGT 3' (Seq.ID.No.: 10); and antisense: 5'CTGAGGAGGAAAGAGCGCTC3' (Seq.ID.No.: 11); to generate a PCR fragment. PCR fragments were purified on Centriflex columns (ACGT, Inc.) and sequenced directly using POP-6 polymer on an ABI Prism 310 Automated Sequencer (Perkin-Elmer). The mutation was verified by restriction endonucleases digestion using HgaI, according to the manufacturer's specifications (New England Biolabs).

To verify that the missense mutation was not a normal polymorphic variant, the mutation was screened for by a combination of heteroduplex analysis. Ganguly, et al., 1993, *Proc Natl. Acad Sci. USA* 90:10325. Direct sequencing and restriction digestion in a control population consisting of 142 unrelated, unaffected individuals, 87 of whom were of

Pakistani origin. No evidence was found for the mutant allele in 284 unrelated, unaffected alleles, 174 were ethnically matched for the congenital alopecia universalis family. The absence of the mutant allele in control individuals, together with the non-conservative nature of the amino acid substitution, strongly suggests that this mutation in the human *hairless* gene underlies the AU phenotype in this family.

The *hairless* mouse *hr/hr*, was first described in the literature in 1924 (Brooke, 1924, *J. Hered.* 15:173), and was later found to have arisen from spontaneous integration of an endogenous murine leukemia provirus into intron 6 of the *hr* gene (Stoye, et al., 1988, *Cell* 54:383), resulting in aberrant splicing and only about 5% normal mRNA transcripts present in *hr/hr* mice. Cachon-Gonzalez, et al., 1994, *Proc. Natl. Acad. Sci. U.S.A.*, 91:7717. The protein encoded by the *hr* gene contains a single zinc finger domain, and is therefore thought to function as a transcription factor (*Id.*), with structural homology to the GATA family (Arceci, et al., 1993, *Mol. Cell. Biol.* 13:2235) and to TSGA, a gene expressed in rat testis (Morrissey, et al., 1980, *J. Immunol.* 125:1558). In addition to the total body hair loss bearing striking resemblance congenital alopecia universalis, the *hr/hr* mouse exhibits a number of phenotypic effects not observed in the AU family, including defective differentiation of thymocytes (*id.*), as well as a unique sensitivity to UV and chemically induced skin tumors (Gallagher, et al., 1984, *J. Invest. Dermatol.* 83:169). Surprisingly, *hr* is not expressed in thymus, yet it is highly expressed in the cerebellum of developing post-natal rat brain, where its significance remains unknown. Thompson, 1996, *J. Neurosci.* 16:7832. *hr* is directly induced by thyroid hormone receptor, which regulates its expression in CNS development, but not in skin. Thompson, et al., 1997,

*Proc. Natl. Acad. Sci. U.S.A.* 94:8526. The phenotypic restriction of the human *hr* mutation to the hair follicle in congenital alopecia universalis family members may reflect the phenomenon of tissue-specific sensitivity of mutations in transcription factor genes described in other disorders, in which there exists a propensity for malfunction in some target organs, and not in others, thus not reflecting the complete expression pattern of the gene. Semeza, 1994, *Hum Mutat.* 3, 180 (1994); Latchman, 1996, *New Engl. J. Med.* 28:334; Engelkamp and van Heyningen, 1996, *Curr. Opin Genet. Dev.* 6:334. The segregation of the congenital alopecia universalis mutation in a recessive fashion in the family suggests that the mutant allele does not function through haplo-insufficiency, nor does it elicit a dominant-negative effect, since heterozygous carriers appear unaffected. Instead, it is proposed that in congenital alopecia universalis, this mutation disrupts a potential activation domain with restricted specificity in the skin, whereas the *hr/hr* mouse displays a more pleiotropic defect due to the near absence of *hr* mRNA and protein.

Example 2: Antibodies specific for the human hairless protein.

Antibodies which bind to the Human hairless protein are prepared using an intact polypeptide or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or a peptide used to immunize an animal can be derived from translated cDNA or chemical synthesis which can be conjugated to a carrier protein, if desired. Such commonly used carriers which are chemically coupled to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat or a rabbit).

If desired, polyclonal or monoclonal antibodies can be



further purified, for example, by binding to and elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (See for example, Coligan, et al, Unit 9, Current Protocols in Immunology, Wiley Interscience, 1994, incorporated herein by reference).

It is also possible to use the anti-idiotypic technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody.

More recently, techniques to make humanized and human antibodies to proteins have been described and are useful to the production of antibodies to an Human hairless protein. For example, methods for obtaining human or humanized antibodies may also be used to obtain antibodies of the present invention. Such methods are described in, for example, EP 7655172, EP 671951, US 5,565,332, and EP 616640.

For example, antibodies may be generated by using a computer-selected peptide such as amino acids of hairless mouse having identity with at least 12 human hairless amino acids.

PCR techniques may also be used to subclone an EcoR1/Not1 fragment corresponding to exons 13-19 of hairless into the EcoR1/Not1 site of pGEX4T. This permits the production of copious quantities of the carboxyterminus of hairless in E. Coli. Protein then may be purified using affinity chromatography, and the GST tag will be cleaved from hairless by thrombin. The protein will be purified, and injected into rabbits.

The carboxy-terminal region of the Human hairless protein into an E. Coli may also be subcloned and an expression vector, allowing the expression of a recombinant fusion protein between the Human hairless protein and a GST tag identified. The presence of the GST tag allows the easy purification of the protein by affinity chromatography. Nilsson et al, 1985, EMBOJ, 4, 4,1075. The GST tag will then be removed with thrombin, and the resultant untagged Human hairless protein will be injected into rabbits. Sera will be by Western analysis against E. Coli expressed protein and extracts prepared from normal and mutant mice.

Example 3: Identification Of Regulatory Sequences and Targets of Human hairless protein.

To identify factors that modulate the expression of the Human hairless protein gene in normal fibroblasts, keratinocytes and other types of skin and hair follicle cells, the minimal 5' upstream regions of the Human hairless protein promoter required for faithful and abundant expression in mouse dermal keratinocytes may be identified. These regions can then be used to identify, clone and characterize transacting factors that bind to these regions.

More specifically, methods which are well known to those skilled in the art may be used to obstruct vectors containing various segments of the Human hairless protein promoter cloned 5' upstream of a reporter gene, such as beta galactosidase. Transgenic mice may be constructed that possess these DNAs, and sequences that confer appropriate epidermal expression to the beta galactosidase reporter gene will be identified. Byrne et al., Development 120,2369 (1994). Based on these results, trans-acting factors that bind these sequences and activate expression will be identified and cloned using standard gel shift, DNA footprinting, DNA mutagenesis, transfection and screening techniques. Leask et al., Genes and Development 4, 1985 (1990); the techniques described in Maniatis, et al., 1989, Molecular Cloning a Laboratory Manual, Cold Spring Harbor

Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y.

5 To identify genes upregulated or downregulated by the Human hairless protein and are candidates for a diffusible protein expressed in skin cells that induces hair follicle formation, subtractive DNA hybridization and differential display techniques may used, as well as CASTing to look for  
10 the hairless DNA binding site, and use this to identify new genes, followed by analysis of these cDNAs in vitro and in vivo.

For example, fibroblasts and keratinocytes from wild-type  
15 and hairless (hr/hr) mice may be cultured. Using standard procedures, RNA will be extracted, cDNA will be prepared from these sources and cDNAs from mutant tissue will be removed from wild-type tissue. Chomezynski and Sacchi. 1987, Anal. Biochem. 162,156; the techniques described in Maniatis et al., 1989, Molecular Cloning a Laboratory Manual, Cold  
20 Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y. Alternatively, messages present in significantly higher abundance in wild-type tissue will be identified by differential display Liang  
25 and Pardee, eds., 1997. Differential Display Methods and Protocols, Human Press, Totowa, N.J. Messages present only or predominantly in wild-type fibroblasts will be selected for further analysis. Tissue restricted expression of the  
30 proteins encoded by these cDNAs will be verified by Northern blotting and *in situ* hybridization. Functionally of these proteins will be determined by expressing them into mutant fibroblasts, either *in vitro* or *in vivo*, for example using adenoviral expression vectors. Kashiwagi et al., 1997,  
35 Development Biology 189,22. Following identification of those nucleotides which encode proteins that rescue the hr/hr phenotype, and are therefore downstream targets of the Human hairless protein and necessary for its function, such

proteins and nucleotides will be assayed further.

Example 4: Antisense Regulation of Human hairless protein Activity

5 A therapeutic approach using antisense to human hairless can be used to directly interfere with the translation of Human hairless protein messenger RNA into protein is possible. For example, antisense nucleic acid or ribozymes could be used to bind to the Human hairless protein mRNA or to cleave  
10 it. Antisense RNA or DNA molecules bind specifically with a targeted gene's RNA message, interrupting the expression of that gene's protein product. See, Weintraub, Scientific American, 262:40, 1990. The antisense molecule binds to the messenger RNA forming a double stranded molecule which  
15 cannot be translated by the cell. Antisense oligonucleotides of about 15-25 nucleotides are preferred since they are easily synthesized and have an inhibitory effect just like antisense RNA molecules. Molecular analogs of oligonucleotide may also be used for this purpose and  
20 have the added advantages of stability, distribution or limited toxicity that are advantageous in a pharmaceutical product. In addition, chemically reactive groups, such as iron-linked oligonucleotide, causing cleavage of the RNA at the site of hybridization. These and other uses of  
25 antisense methods to inhibit the *in vitro* translation of genes are well known in the art (Marcus-Sakura, Anal., Biochem, 172:289, 1988).

30 Delivery of antisense therapies and the like can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferable, an RNA virus such as a retrovirus. Preferably,  
35 the retroviral vectors is a derivative of a murine or avian retrovirus. Examples retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV). Harvey murine

sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), an dRous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a polynucleotide sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a desired specific target cell, for example, can make the vector target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a protein or proteins such that the desired ligand is expressed on the surface of the viral vector. Such ligand may be glycolipid carbohydrate or protein in nature. Preferred targeting may also be accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the antisense polynucleotide.

Since recombinant retroviruses are typically replication defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Helper cell lines which have deletions of the packaging signal may used. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate transfection. These cells are then transfected  
5 with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

With respect to colloidal dispersion systems as a method for  
10 accomplishing targeted delivery of an antisense polynucleotides, these systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal  
15 system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0  
20  $\mu$ m can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., Trends Biochem. Sci., 6:77, 1981). In addition to  
25 mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present:  
(1) encapsulation of the genes of interest with high efficiency while not comprising their biological activity;  
30 (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., Biotechniques, 6:682,  
35 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-

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temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations. Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes has been classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs or cells types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of liposomal targeted delivery system, lipid groups can be incorporated in the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand. In general, the

compounds bound to the surface of the targeted delivery system to find and "home in" on the desired cells. A ligand may be any compound of interest which will bind to another compound, such as growth factor.



Second Series of Experiments

A number of genetically determined primary T cell immunodeficiencies have been described in which T lymphocytes are present in normal or reduced number, but specific T cell functions are dysregulated. We studied a family with congenital alopecia and severe T-cell immunodeficiency, whose clinical findings were reminiscent of the nude mouse phenotype. We found suggestive evidence of linkage to the *whn* locus on human chromosome 17 ( $Z_{\max}=1.32$ ), and identified a homozygous nonsense mutation in the human *whn* gene in affected individuals. The human *whn* gene encodes a forkhead/winged helix transcription factor with restricted expression in the thymus, epidermis, and hair follicle.

In the past several years, extraordinary progress has been made in understanding the molecular basis of genetic disorders resulting in primary immunodeficiencies in humans, and in many cases has provided significant insights into crucial steps of lymphocyte development and immune system function in general. Fischer, A., et al. (1997) *Annu. Rev. Immunol.* 15:93; Kokron, C.M., et al. (1997) *Clin. Immunol.* 17:109; Fischer, A. (1996) *Curr. Opin. Immunol.* 8:445; Arnaiz-Villena, A., (1992) *Immunol* 13:259. Severe combined immunodeficiencies (SCID) represent the most severe group of primary immunodeficiencies, whose overall frequency is about one in 75,000 births. Fischer, A., et al. (1997) *Annu. Rev. Immunol.* 15:93; Kokron, C.M., et al. (1997) *Clin. Immunol.* 17:109; Fischer, A. (1992) *Curr. Opin. Immunol.* 8:445; Arnaiz-Villena, A., (1992) *Immunol* 13:259. These inherited diseases include a wide spectrum of clinically and genetically heterogeneous disorders affecting either the differentiation or the cell activation process. The most severe form is usually lethal in the first year of life due to severe immunological impairment and life threatening infections. In contrast, the clinical course of a few cases of SCIDs with a predominant qualitative T-cell defect is milder, and is characterized by a wide range of clinical features caused either directly or indirectly by the

underlying disease. The clinical and immunological heterogeneity of the SCIDs reflects an underlying genetic heterogeneity. There are currently seven different forms of SCID that are grouped according to pattern of inheritance, disease phenotype and in some, the identification of underlying gene mutations. Fischer, A., et al. (1997) *Annu. Rev. Immunol.* 15:93; Kokron, C.M., et al. (1997) *Clin. Immunol.* 17:109; Fischer, A. (1996) *Curr. Opin. Immunol.* 8:445; Arnaiz-Villena, A., (1992) *Immunol* 13:259. Mutations in the common  $\gamma$ -chain gene ( $\gamma$ c) of several cytokine receptors have been reported in X-linked SCID with (T-)(B+) phenotype, while mutations in the JAK-3 kinase gene have been described in the autosomal recessive form. Fischer, A., et al. (1997) *Annu. Rev. Immunol.* 15:93; Kokron, C.M., et al. (1997) *Clin. Immunol.* 17:109; Fischer, A. (1996) *Curr. Opin. Immunol.* 8:445; Arnaiz-Villena, A., (1992) *Immunol* 13:259; Noguchi, M., et al. (1993) *Cell* 73:147; Macchi, P., et al. (1995) 377:65. Evidence is emerging that these molecules are of critical importance in either thymic maturation and T-lymphocyte development or cell activation processes. Boussiotis, V.A., et al. (1994) *Science* 266:1039; Baird, A.M. (1998) *J. Leukoc. Biol.* 63:669. Null mutations in the Rag-1 or Rag-2 genes have been described in an autosomal recessive SCID with a (T-)(B-) phenotype. Fischer, A., et al. (1997) *Annu. Rev. Immunol.* 15:93; Kokron, C.M., et al. (1997) *Clin. Immunol.* 17:109; Fischer, A. (1996) *Curr. Opin. Immunol.* 8:445; Arnaiz-Villena, A., (1992) *Immunol* 13:259; Schwartz, K. Et al., (1996) *Science* 274:97. In all these forms, natural killer (NK) cells are undetectable. Recently, partial loss-of-function mutations in the Rag-1 and Rag-2 genes have been implicated in Omenn syndrome, a leaky (T-)(B-)SCID phenotype characterized by hypereosinophilia, erythrodermia, and severe liver disease. Fischer, A., et al. (1997) *Annu. Rev. Immunol.* 15:93; Kokron, C.M., et al. (1997) *Clin. Immunol.* 17:109; Fischer, A. (1992) *Curr. Opin. Immunol.* 8:445; Arnaiz-Villena, A., (1992) *Immunol* 13:259; Villa, A., et al. (1998) *Cell* 93:885; Romagnani, S. (1996)

- Clin. Immunol. Immunopathol.* 80:225; Romagnani, et al. (1997) *Int. Arch. Allerg. Immunol.* 113:153. In this form, lymphocytes are predominately of the Th2 phenotype and exhibit a limited usage of the TCR repertoire. Fisher, A., et al. (1997) *Annu. Rev. Immunol.* 15:93; Kokron, C.M., et al. (1997) *Clin. Immunol.* 17:109; Fischer, A. (1992) *Curr. Opin. Immunol.* 8:445; Arnaiz-Villena, A., (1992) *Immunol* 13:259; Villa, A., et al. (1998) *Cell* 93:885; Romagnani, S. (1996) *Clin. Immunol. Immunopathol.* 80:225; Romagnani, et al. (1997) *Int. Arch. Allerg. Immunol.* 113:153. Remarkable progress has also been made in the study of SCID with predominant T-cell defect in which T lymphocytes are present in normal or reduced number, but specific T cell function(s) are partially dysregulated, referred as qualitative disorders. The genetic bases of relatively few types of these forms of SCID are known, including partial CD3 $\epsilon$  expression deficiency and CD3 $\gamma$  subunit expression deficiency. Fischer, A., et al. (1997) *Annu. Rev. Immunol.* 15:93; Kokron, C.M., et al. (1997) *Clin. Immunol.* 17:109; Fischer, A. (1992) *Curr. Opin. Immunol.* 8:445; Arnaiz-Villena, A., (1992) *Immunol* 13:259; Arnaiz-Villena, A. Et al., (1992) *N. Engl. J. Med.* 327:529; Soudais, C., et al. (1993) *Nature Genet.* 3:77; Arpaia, E. Et al. (1994) *Cell* 76:1.
- Alterations in the signal transduction process through the TCR/CD3 complex(ZAP-70) lead to a SCID phenotype predominantly affecting CD8 $^{+}$  lymphocytes. Fischer, A., et al. (1997) *Annu. Rev. Immunol.* 15:93; Kokron, C.M., et al. (1997) *Clin. Immunol.* 17:109; Fischer, A. (1992) *Curr. Opin. Immunol.* 8:445; Arnaiz-Villena, A., (1992) *Immunol* 13:259; Elder, M.E., et al. (1994) *Science* 264:4596; Chan, A.C., et al. (1994) *Science* 264:4599. Mutations in the human equivalent of the mouse beige gene underlie the cytotoxic T lymphocyte and NK deficiency typical of Chediak-Higashi syndrome. Nagle, D.L., et al. (1996) *Nature Genet.* 14:307. However, the molecular basis of many of these cases remains to be determined.

Recently, the simultaneous occurrence of severe functional T-cell immunodeficiency, congenital alopecia, and nail dystrophy (MIM 601705) in two female siblings from a consanguineous Italian family was reported as a syndrome for the first time.

5 Pignata, S. (1996) *Am.J. Med. Genet.* 65:167. At birth, both children presented with a complete absence of hair and dystrophic nails, and no thymic shadow was evident in either child upon X-ray examination. In addition, the first affected child revealed a striking impairment of T-cell function

10 shortly after birth, and rapidly developed a clinical phenotype characterized by erythrodermia, persistent diarrhea, failure to thrive, and hypereosinophilia, reminiscent of Omenn syndrome. She died at the age of 12 months of resistant bronchopneumonia after recurrent infections. The second

15 affected child also showed immunological abnormalities at the age of one month, and later, she presented with a severe impairment of humoral and cell-mediated immunity and suffered from recurrent respiratory tract infections. At the age of 5 months, the patient received an HLA-identical total bone

20 marrow transplant (BMT) from her unaffected brother, following only two doses of antilymphocyte serum, and no immunosuppressive therapy or immunodepletion by irradiation. Bone marrow transplantation led to full immunological reconstitution in the patient, whereas the generalized

25 alopecia and the nail dystrophy are still present. The persistence of the generalized alopecia following successful BMT suggested that it was not acquired in nature, but instead was related to the immunodeficiency. The severe immunodeficiency in both children was characterized by a

30 decrease of mature T lymphocytes, mainly due to a low number of helper T cells, whereas the number of suppressor/cytotoxic T cells was relatively normal. However, the patients had a normal number of overall circulating lymphocytes due to the predominance of mature B-lymphocytes. In contrast to SCID

35 patients with JAK-3 and  $\gamma$ c mutations, NK cells in both patients were unaffected. In the two children studied, the T-cell immunodeficiency was qualitative in nature, in that peripheral blood T cells failed to undergo mitogen-induced

activation and cell-cycle progression. Pignata, C. Et al. (1996) *Am. J. Med. Genet.* 65:167. Although the B cell machinery appeared to be intact, insofar as allohemagglutinins were detected, as expected, B lymphocytes were unable to produce specific antibodies against T dependent antigens. Pignata and colleagues recognized that the association between alopecia and immunodeficiency in their patients was not serendipitous, and might in fact be related to a common gene defect. Further, they speculated that the clinical symptoms in both patients were reminiscent of the nude mouse phenotype, which is associated with congenital alopecia and athymia, causing severe immunodeficiency due to a lack of T-lymphocytes, Flanagan, S.P. (1966) *Genet. Res.* 8:295; Pantelouris, E. (1968) *Nature* 217:370; Gershwin, M.E. (1977) *Am. J. Pathol.* 89:809; Festing, M.F.W., et al. (1978) *Nature* 274:365; Sundberg, J.P. (1994) *Handbook of Mouse Mutations with Skin and Hair Abnormalities* (CRC Press, Boca Raton) p 379-389, and resulting from mutations in the *whn* gene (winged-helix-nude, *Hfh 11<sup>nu</sup>*), which encodes a forkhead/winged helix transcription factor with restricted expression in thymus and skin. Nehls, et al., 1994, *Nature* 372:103; Segre, et al., 1995, *Genomics* 28:549; Huth, et al. 1997, *Immunogenetics* 45:282; Hofmann, et al., 1998, *Genomics* 52:197; Schorpp, et al., 1997, *Immunogenetics* 46:509. Linkage analysis was performed using microsatellite markers near the human *whn* locus chromosome 17, as deduced from the published map of the syntenic region on mouse chromosome 11. Nehls, et al., 1994, *Nature* 372:103; Segre, et al., 1995, *Genomics* 28:549; Huth, et al. 1997, *Immunogenetics* 45:282; Hofmann, et al., 1998, *Genomics* 52:197; Schorpp, et al., 1997, *Immunogenetics* 46:509.

DNA samples from the original family members from a small village in southern Italy (Figure 7) were obtained. Each family member from whom DNA was obtained was examined and the clinical phenotype of the affected individual is characterized

by a severe, complete alopecia involving the scalp, eyebrows and eyelashes. Four children in a different branch of the family were reported anamnesticly to have been affected with the same disorder, and died in early childhood. Linkage analysis was performed using microsatellite markers near the whn locus on chromosome 17, as deduced from the published map of the syntenic region on mouse chromosome 11. Blood samples were also collected from 17 members of the family, according to local informed consent procedures. DNA was isolated from PBMCs prepared in TriReagent (Sigma) according to the manufacturer's recommendations. Screening of all regions of chromosome 17 showing homozygosity in affected family members was carried out using primers obtained from Research Genetics, Inc., or in the Genome Data Base (<http://www.gdb>). Analysis of microsatellite markers consisted of end-labeling one primer using  $\gamma^{32}\text{P}$  dATP, a PCR reaction consisting of 7 minutes at 95° C, followed by 27 cycles of 95° C, 1 minute, 55° C, 1 minute; 72° C, 1 minute; and electrophoresis in a 6% polyacrylamide gel (Sequa-gel, Action Scientific). Microsatellite markers were visualized by exposure of the gel to autoradiography, and genotypes were assigned by visual inspection. DNA was collected from both patients with congenital alopecia (Individuals V-2 and V-3 in Figure 7), their brother (Individual V-1 in Figure 7). For the second born patient, DNA samples were available from before and after the bone marrow transplantation. In addition, DNA was collected from 11 unaffected family members in the extended pedigree, which contained a single consanguinity loop between the paternal grandfathers of the probands (Figure 7). Genotyping with three markers, D17S798, D17S1800 and D17S1857, revealed a homozygous haplotype in both affected patients. Both parents were heterozygous for the same haplotype, as were several clinically unaffected relatives (Figure 7). Two point and multipoint analyses were performed on the markers D17S798, D17S1857 and D17S1800. The genetic model assumed for the analysis was a fully penetrant recessive model with a disease

allele frequency of 0.0001. Marker allele frequencies were estimated using founders' alleles. Boehnke, 1991, *Am.J.Hum.Genet.* 48:22. Map positions and intermarker distances were determined using the Marshfield website (www.marshmed.org/genetics/). All lod scores were calculated using the LINKAGE programs ILINK and LINKMAP. Lathrop, et al., 1984, *Proc. Natl. Acad. Sci. USA* 81:3443; Schaffer, 1996, *Hum. Hered.* 46:226. The maximum two point lod score was 1.32, observed at both D17S798 and D17S1800. With multipoint analysis, the lod score at all markers was 1.32, suggestive of linkage of the disease phenotype in the family with markers near the *whn* gene. Multipoint analysis did not improve the scores at markers D17S798 and D17S1800 as the markers were already fully informative in this family. The observation of an unaffected individual with two recombination events allowed localization of the *whn* gene within a 10.4 cM interval between the markers D17S98 and D17S1857 (Figure 7).

Primer pairs were developed to amplify all exons and flanking splice sites based on the cDNA structure of the human sequence, Schorpp, et al., 1997, *Immunogenetics* 46:509, (GenBank accession number Y11739). A mutation detection strategy was developed based on PCR amplification of all *whn* exons. For amplification of exon 5 of the *whn* gene in this study, the following primers were used:

Exon 5F: 5'CTTCTGGAGCGCAGGTTGTC3' (Seq.ID.No.:12)

Exon 5R: 5'TAAATGAAGCTCCCTCTGGC3' (Seq.ID.No.:13)

PCR amplification resulted in a product 184 bp in size, containing 7 bp of intron 4, 131 bp of exon 5, and 46 bp of intron 5. PCR was carried out on genomic DNA from the patients, all family members, and the control individuals according to the following program: 95° C for 5 minutes; followed by 35 cycles of 95° C for 45 seconds, 55° C for 45 seconds, and 72° C for 1 minute; followed by 72° C for 7 minutes, in a Stratagene RoboCycler Gradient 96 thermal cycler

(Stratagene, LaJolla, CA). PCR products were run on a 2% agarose gel and purified in a first step using the High Pure PCR product purification kit (Boehringer Mannheim). In a second step, PCR fragments were purified on Edge Centriflex columns (Edge BioSystems, Gaithersburg, MD) and sequenced directly with POP-6 polymer using an ABI Prism 310 Genetic Analyzer from Applied Biosystems Inc. (Perkin Elmer). The mutation was verified by restriction enzyme digestion using *BsrI*, according to the manufacturer's guidelines (New England Biolabs). In both patients, direct sequencing analysis of the PCR fragment containing exon 5 of the *whn* gene revealed a homozygous C-to-T transition (figure 8a) at nucleotide position 792 of the *whn* cDNA (numbered according to GenBank #Y11739). This base substitution leads to a nonsense mutation at amino acid position 255 of the protein, converting an arginine residue (CAG) to a premature termination codon (TAG), designated R255X. In addition to direct sequencing analysis, restriction digestion with the endonuclease *BsrI* was used to confirm the sequence variation in exon 5 (Figure 8B) (see method above). Genotyping of the extended family members revealed eight individuals who are clinically unaffected heterozygous carriers of the mutation, consistent with the segregation of the disease-associated haplotype (Figure 7). The mutation was not identified in 102 unaffected, unrelated Northern European control individuals, indicating that R255X is not a common polymorphism. The nonsense mutation identified in this invention results in a premature termination codon (PTC) at amino acid residue 255 of the *whn* protein, within exon 5. In general, PTCs result in dramatic reductions in the steady-state level of cytoplasmic mRNA, due to nonsense-mediated mRNA decay, Cooper, 1993, *Ann. Med.* 85:11; Maquat, 1995, *RNA* 1:453, thereby predicting an absence of functional protein.

Since the proband's BMT was derived from her brother, the



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leukocyte DNA from the proband and her brother were examined before and after grafting for the presence of fraternal chimerism. For determination of the X and Y chromosome complement of the family members, gender determination was performed by restriction analysis of simultaneously amplified ZFX and ZFY sequences as previously described in Chong, et al., 1993, *Hum. Molec. Genet.* 2:1187. Genotyping revealed that the brother was a carrier of the mutant maternal *whn* allele and the wild-type paternal *whn* allele (Figure 7). Genotyping of the proband before BMT revealed that her leukocyte DNA was homozygous for the mutant haplotype only (Figure 7). Four years after BMT, evidence for chimerism in her leukocyte DNA was ascertained by detection of the haplotype specific for the wild-type paternal *whn* allele as well as the mutant allele. Further, gender determination using primers specific for the X and Y chromosomes revealed that prior to the BMT, the proband's peripheral blood leukocyte DNA (female) was genotypically XX and the brother's DNA (male) was XY (Figure 8C). After the BMT, however the proband's leukocyte DNA was found to be xy, providing evidence for long-term engraftment and expansion of the BMT graft from the donor brother.

Three independent analyses of mRNA transcripts in a variety of human tissues were performed to determine the expression of patterns of *whn* in a variety of human tissues. The Human RNA Master Dot Blot (#7770-1) containing mRNA from 24 different human tissues was obtained from Clontech and hybridized using ExpressHyb solution according to the manufacturer's recommendations, with a probe spanning 482 bp of the *whn* cDNA (nucleotides 1185-1646). The Human Multiple Tissue Northern Blot (MTN) II (#7759-1) containing 2µg poly A+ mRNA from eight tissues and the Human Immune System II Multiple Tissue Northern Blot containing six tissues, were obtained from Clontech (Palo Alto, CA) and hybridized with a random primed radiolabelled probe corresponding to nucleotides 18-729 of

human whn (GenBank #Y11739). Total RNA was extracted from cultured Swiss 3T3 mouse manufacturer's instructions (Qiagen Rneasy Kit, Santa Clarita, CA) and 10µg RNA from cultured fibroblasts and keratinocytes, Rheinwald and Green, 1975, *Cell* 5 6:331; Simon and Green, 1985, *Cell* 40:677, were electrophoresed according to standard techniques. Sambrook, et al., 1989, *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2). All northern blots were hybridized with the same probe at 42° C in 10 50% formamide, and final washes were performed at 65° C in 0.2xSSC, 0.1% SDS. *In situ* hybridization was performed as previously described, Panteleyev, et al., 1997, *J. Invest. Dermatol.* 108:324 in 0.5 µ sections of paraffin embedded normal human scalp skin from a 35 year old female donor. 15 Briefly, deparaffinized and deproteinized sections were acetylated in acetic anhydride solution (EM Science, Gibbstown, NJ) and then dehydrated. Prehybridization was performed in humidified chambers at 50°C with a mixture containing 50% deionized formamide (EM Science, Gibbstown, 20 NJ). Hybridization with 50 ng/section of freshly denatured cRNA probes was performed at 50 C for 17h in the same humidified chambers. The cRNA probe for whn was synthesized from genomic DNA using sequences contained within exon 8 of the human whn cDNA (GenBank #Y11739). The forward primer (nt 25 1284-1305) was 5'CTCTCCCCACCACTGCACTCACT3' (Seq.ID.No.:14) and the reverse primer (nt 1597-1618) was 5'TCCAGGTCAGTGCCAAGGTCTC3' (Seq.ID.No.:15). The human whn sense-probe was used as a negative control. Sections were washed after hybridization at 50 C under high stringency 30 conditions for 5h. Prior to immunodetection of the *in situ* hybridization signal, the slides were incubated with normal sheep serum (Sigma, St. Louis, MO, USA) in the presence of levamisole (Sigma, St. Louis, MO, USA) and blocking solution (DIG Nucleic Acid Detection Kit, Boehringer-Mannheim, 35 Mannheim, FRG). Incubation with sheep alkaline phosphatase

labeled anti-digoxigenin antibodies (DIG Nucleic Acid Detection Kit, Boehringer-Mannheim, Mannheim, FRG) was performed for 3 hours in humidified chambers at room temperature. The slides were stained by incubation in  
5 nitroblue tetrazolium and 6-chloroindolylphosphate solution (Boehringer-Mannheim, Mannheim, FRG) for 16-20h in complete darkness at room temperature. After short washing, the sections were mounted in Kaiser's glycerol gelatin (Merck, Darmstadt, FRG).

10

Using dot blot hybridization analysis of 24 different human tissues, *whn* was prominently expressed only in fetal and adult thymus, and was essentially negative in all other tissues (Figure 9B). Northern analysis was performed using a multiple  
15 immune tissue blot (six tissues), and a standard multiple tissue blot (eight tissues), and once again, *whn* expression was observed only in the thymus (Figure 9B,C), confirming and extending the expression pattern previously reported. Schroop, et al., 1997, *Immunogenetics* 46:509. Northern analysis using  
20 mRNA from cultured fibroblasts and keratinocytes (Figure 9D) revealed *whn* expression abundantly expressed in epidermal keratinocytes (Figure 9D). Localization of *whn* expression within normal skin was performed by *in situ* hybridization studies. Consistent with the northern analysis, *whn* mRNA is  
25 restricted to the epidermis, and no expression is observed in the dermis (Figure 10A). The basal keratinocytes and the proximal layers of epidermal spinous compartment are highly positive, while in the upper spinous layer, *whn* expression gradually declines. Therefore, *whn* expression spans the  
30 transition from proliferation to terminal differentiation and decreases during later stages of the differentiation program. In the sebaceous glands, *whn* mRNA-positive staining was observed in the thin layer of proliferating reserve cells, but not in the differentiating sebocytes (Figure 10B), and the  
35 sweat gland epithelium was moderately *whn*-positive (Figure

10B).

In the hair follicle, *whn* expression was sharply demarcated in several epithelial cell populations, while the dermal papilla fibroblasts were always *whn* mRNA-negative. The most prominent *whn* mRNA expression was localized in the hair matrix above the level of Auber, and in the innermost cell layer of the outer root sheath (ORS). The line of Auber separates two different matrix cell populations: the lower portion, which contains the undifferentiated proliferating keratinocytes, and the upper portion (or precortex), which consists mainly of differentiating cells. Abell, 1993, in *Disorders of hair growth*, E.A. Olsen, Ed. (McGraw-Hill, Inc.) 1-19. The matrix below the level of Auber is *whn* mRNA-negative, while the differentiating cells above this line are mainly positive with the exception of melanocyte-containing zone just above the dermal papilla (Figure 10C). In addition to differentiating matrix cells, we found prominent *whn* expression in the specific ORS cell layer directly adjacent to the inner root sheath (IRS) (Figures 10B-D) and designated as the "companion layer" or the "innermost cells of the outer root sheath". Ito, 1986, *Arch. Dermatol. Res.* 279:112; Orwin, 1971, *Aust. J. Biol. Sci.* 24:989. This keratinocyte layer is characterized by a unique differentiation pathway, and is morphologically and immunologically distinct from the other ORS keratinocytes, however, its function and origin are still a subject of controversy. Rothnagel and Roop, 1995, *J. Invest. Dermatol.* 104:42S; Panteleyev, et al., 1997, *J. Invest. Dermatol.* 108:324. Weak *whn* expression was found also in the basal keratinocytes of the upper ORS portion starting from the level of sebaceous gland (Figure 10B). In the upper hair follicle infundibulum, this zone of *whn* expression merges with the *whn*-positive basal keratinocytes of the interfollicular epidermis. The IRS was *whn*-negative in both the proximal (Figure 10D) and cornified (Figures 10B,C) portions. Collectively, the patterns of *whn* expression revealed that in the hair matrix,

whn is expressed in differentiating cells; in the interfollicular epidermis, whn is expressed in both the proliferating and differentiating compartments; and in the sebaceous gland, whn is expressed in proliferating cells only.

5 These findings are similar with those for whn expression in mouse interfollicular epidermis and hair follicle. Taken together, the identification of a pathogenetic mutation in the human whn gene in a family with congenital alopecia with T-cell immunodeficiency, and the localization of whn expression  
10 to the two human tissues involved in the disease phenotype, strongly implicate whn mutations in the pathogenesis of this disorder.

The protein encoded by the human, mouse and rat nude gene  
15 encodes a member of the forkhead/winged helix class of transcription factors, which are developmentally regulated, and direct tissue-and cell-type specific transcription and cell fate decisions. Lai, et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:10421; Kaufmann and Knochel, 1996, *Mech. Dev.*  
20 57:3. The hallmark of this group of transcription factors is a highly conserved DNA binding domain, encompassing a region of about 110 amino acids containing a modified helix-turn-helix motif, first identified in the *Drosophila* gene forkhead and in rat hepatocyte nuclear factor 3 (HNF-3). In the human,  
25 mouse and rat whn proteins, which are approximately 85% identical, the DNA binding domain spanning amino acid residues 271 to 362, is encoded by exons 5, 6 and 7. Similar to other winged helix proteins, the whn proteins contain an evolutionarily conserved and functionally indispensable acidic  
30 transcriptional activation domain, located in the C-terminus of the protein. This transactivation domain extends from residues 509 to 563, and is encoded by exons 8 and 9. Schuddekopf, et al., 1996, *Proc. Natl. Acad. Sci. USA* 93:9661; Schlake, et al., 1997, *Proc. Natl. Acad. Sci. USA* 94:3842.  
35 The nonsense mutation in the family under study resides in exon 5, upstream of both the DNA-binding and the

transactivation domain of *whn* genes consist of eight coding exons and utilization of two alternative first (non-coding) exons in a tissue-specific manner. Heterologous reporter assays have demonstrated promoter activity upstream of both first exons, and although both promoters are active in the skin at variable levels, only the upstream promoter is active in the thymus, suggesting that *whn* may be subject to complex cell-type specific transcriptional regulation. Schorpp, et al., 1997, *Immunogenetics* 46:509. *Whn* orthologs are highly conserved through evolution, and have been cloned from eight different species, including human, mouse, rat, pufferfish, zebrafish, shark, lamprey and amphioxus. The extent of homology correlates with evolutionary distance, yet the conservation between the two most distant relatives, human and amphioxus, is nearly 80% identical at the amino acid level, demonstrating a remarkable degree of conservation over more than 500 million evolutionary years. The function of *whn* in agnathans (lamprey) and cephalochordates (amphioxus), which do not have hair nor a thymus, and bony fish (zebrafish and pufferfish), which do not have hair but do have a thymus, is currently unknown, however, it may perform a similar function in diverse types of epithelia through vertebrate evolution.

In mammals, *whn* is expressed specifically in the epithelial cells of the skin and thymus, where it appears to play a critical role in maintaining the balance between growth and differentiation, Nehls, et al., 1996, *Science* 272:886; Brissette, et al., 1996, *Genes & Dev.* 10:2212, since mutations at the nude locus disrupt both hair growth and thymic development. The main function of the thymus is to generate and select a diverse repertoire of T cells which display self-tolerance and restriction to the host's major histocompatibility complex. Recent evidence has underscored the importance of the thymic microenvironment in determining the T cell repertoire, since both positive and negative

selection of developing T cells depends on cell-cell interactions with the thymic epithelium. In athymic nude mice and transgenic *Hfh 11<sup>nu</sup>* knock-out mice, the defect has been localized to the thymic microenvironment rather than to an intrinsic defect in the developing T cells themselves. *Whn* is not required for initial formation of the epithelial primordium of the thymus before the entry of lymphocyte progenitors, however, the subsequent differentiation of precursor cells into subcapsular, cortical, and medullary epithelial cells of the mature thymus is critically dependent on *whn* expression. Since *whn* expression persists in thymic epithelial cells throughout life, it may be required not only for the initiation of differentiation but also for maintenance of the differentiated phenotype. For these reasons, it has been speculated that the human *whn* gene might be a good candidate gene for human thymomas and for human thymic dysplasia disorders, such as Nezelof syndrome.

Similar to the thymus, the formation and maintenance of the epidermis and hair follicle also requires a balance between epithelial growth and differentiation. Although nude mice appear to be completely naked, the dermis actually contains a normal number of hair follicles compared to a wild-type mouse, however, the follicles are abnormal and incompletely developed. Kopf-Maier, et al., 1990, *Acta Anat.* 139:178. Although the number and cycling pattern of hair bulbs is normal, impaired keratinization of the hair follicles leads to short, bent hairs that only rarely emerge from the epidermis. Mouse mutations have become an important genetic tool for the identification of specific human genes encoding diseases with clinical features resembling those observed in mutant mice, in particular, for visible phenotypes affecting the fur coat and skin of mice. Sundber and King, 1996, *Invest. Dermatol.* 106:368; Copeland, et al., 1993, *Science* 262. The mapping of inherited human alopecia (MIM 203655) to chromosome 8p21,

using insights provided by the hairless mouse model, enabled cloning of the human hairless gene and identification of mutations in several families with atrichia. The discovery of a human alopecia with mutations in the *whn* gene extends the body of evidence implicating single genes in hair cycle regulation. Sundberg, J.P. and King, L.E. (1996) *J.Invest.Dermatol.* 106:368; Copeland, N.G. (1993) *Science* 262; Nothen, M., et. al. (1998) *Am.J.Hum.Genet.* 62:386.

While the forkhead/winged helix class of transcription factors has been widely studied using mutation of forkhead/winged helix gene was only recently reported. Mutations in the human thyroid transcription factor 2 gene (TTF-2) were identified in a syndrome characterized by thyroid agenesis, cleft palate, bifid epiglottis and spiky hair. Not unlike the athymia observed in the nude phenotype, this disorder results from a complete or partial failure of thyroid gland development. TTF-2 is expressed during the descent of the thyroid primordium from the pharyngeal pouches, then disappears with the onset of thyrocyte differentiation, and reappears later during organogenesis. Clifton-Bligh, R.J. et. al. (1998) *Nature Genet.* 19:399. Patients treated with thyroxine replacement have normal physical growth, sexual development and pituitary function. The observation of phenotypic correction by a simple pharmacologic intervention raises the possibility of modulation of the nude phenotype by exogenous genetic and/or cellular therapies. In support of this notion, therapeutic rescue of the alopecia phenotype in nude mice was recently accomplished using systemic cyclosporin A, Swada, M., et al. (1987) *Am.J.Pathol.* 56:684, and intraperitoneal or subcutaneous administration of recombinant KGF, Danilenko, D.M., et al. (1995) *Am.J.Pathol.* 147:145, presumably by stimulating proliferation of the hair matrix cells and normalizing the keratinization defect. No correction the T cell deficiency was reported in these mice. In addition, transgenic insertion of a cosmid clone containing the wild-type *whn* gene into fertilized Hfh11<sup>nu</sup>/Hfh11<sup>nu</sup> eggs also



- corrected the alopecia phenotype of the resulting mouse, but not the athymic phenotype, suggesting that the upstream thymus-specific *whn* promoter may not have been present in the cosmid clone. Kurooka, H., et al. (1995) *J.Exp.Med.* 181:1223.
- 5 In contrast, transgenic expression of IL-7 in nude mice restored significant populations of T cells, however, also failed to rescue the alopecia phenotype. Rich, B.E. and Leder, P. (1995) *J.Exp.Med.* 181:1223.

**What is claimed is:**

1. An isolated nucleic acid which encodes a wildtype human hairless protein.
2. An isolated nucleic acid which encodes mutant human hairless proteins.
3. The isolated nucleic acid of claim 1 or 2, wherein the nucleic acid is DNA.
4. The isolated nucleic acid molecule of claim 1 or 2, wherein the nucleic acid is RNA.
5. The isolated nucleic acid of claim 3, wherein the nucleic acid is cDNA.
6. The isolated nucleic acid of claim 3, wherein the nucleic acid is genomic DNA.
7. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises a nucleic acid having a sequence substantially the same as the sequence designated SEQ. ID. No.: 1.
8. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises a nucleic acid having a sequence substantially the same as the sequence designated SEQ. ID. No.: 1 and wherein a G to A transition occurs at the first base of a threonine (T) residue at position 1022 (ACA) converting the threonine residue to an alanine (A) residue as indicated for the human sequence (H) in Figure 1.
9. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises a nucleic acid having a sequence substantially the same as the sequence designated SEQ. ID. No.: 1 and wherein a nucleotide transition occurs at

a threonine (T) residue at position 1022 (ACA) converting the threonine residue to an alanine (A) residue as indicated for the human sequence (H) in Figure 1.

- 5      10. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises a nucleic acid having a sequence substantially the same as the sequence designated SEQ. ID. No.: 1 and wherein a nucleotide transition occurs at a threonine (T) residue at position 1022 (ACA) converting the threonine to an amino acid residue other than alanine.
- 10
11. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises a nucleic acid having a sequence substantially the same as the sequence designated SEQ. ID. No.: 1, wherein a nucleotide transition occurs at a residue for hairlessness converting the amino acid residue in the region to a different amino acid.
- 15
12. A vector comprising the nucleic acid molecule of claim 1.
- 20
13. The vector of claim 12, wherein the vector is a virus, cosmid, yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), bacteriophage or a plasmid.
- 25
14. A host vector system for the production of a human hairless protein which comprises the vector of claim 12 in a suitable host.
- 30
15. The host vector system of claim 14, wherein the suitable host is a bacterial cell or a eukaryotic cell.
16. The host vector system of claim 14, wherein the suitable host is a mammalian cell, yeast or insect cell.
- 35
17. A nucleic acid probe comprising a nucleic acid of at least 11 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within the nucleic

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acid of claim 1 or 2.

18. The nucleic acid probe of claim 17, wherein the nucleic acid probe is DNA.
19. The nucleic acid probe of claim 17, wherein the nucleic acid probe is RNA.
20. A nucleic acid, wherein the nucleic acid is the antisense of the nucleic acid of claim 1 or a portion thereof.
21. An isolated wildtype human hairless protein.
22. An isolated wildtype human *whn* protein.
23. The isolated wildtype human *whn* protein of claim 22, having a homozygous arginine to a premature termination codon transition (C-to-T) at nucleotide position 792 leading to a mutation at amino acid position 255 of the protein as indicated in Figure 8A.
24. An isolated mutant human hairless protein.
25. The protein of claim 24, having substantially the same amino acid sequence as the human amino acid sequence (H) shown in Figure 4 (SEQ.ID.NO.: 3).
26. The protein of claim 24, having substantially the same amino acid sequence as the human amino acid sequence (H) shown in Figure 4 (SEQUENCE ID NO.: 3) except the threonine (T) at position 1022 is replaced by alanine (A) and is designated herein as SEQ.ID.NO.: 4.
27. The protein of claim 24, having substantially the same amino acid sequence as the human amino acid sequence (H) shown in Figure 4 (SEQUENCE ID NO.: 3) except the threonine (T) at position 1022 is replaced by an amino acid other than alanine.

28. A method of isolating a nucleic acid encoding a wildtype human hairless-related protein in a sample containing nucleic acid which comprises
- 5 (a) contacting the nucleic acid in the sample with the nucleic acid probe of claim 17, under conditions permissive to the formation of a hybridization complex between the nucleic acid probe and the nucleic acid;
- 10 (b) isolating the complex formed; and
- (c) separating the nucleic acid probe and the nucleic acid, thereby isolating the nucleic acid encoding a wildtype human hairless-related protein in the sample.
- 15 29. The method of claim 28, step (a) further comprising
- (a) amplifying the nucleic acid in the sample under conditions permissive to polymerase chain reaction; and
- 20 (b) detecting the presence of a polymerase chain reaction product, the presence of polymerase chain reaction product identifying the presence of a nucleic acid encoding a human hairless-related protein in the sample.
- 25 30. The nucleic acid isolated by the method of claim 28.
31. The method of claim 29, wherein the detection of the polymerase chain reaction product comprises contacting
- 30 the nucleic acid molecule from the sample, wherein the nucleic acid probe is labeled with a detectable marker.
32. The method of claim 31, wherein the detectable marker is a radiolabeled molecule, a fluorescent molecule, an
- 35 enzyme, a ligand, or a magnetic bead.
33. A method for identifying a compound which is capable of enhancing or inhibiting expression of a human hairless

protein comprising:

- 5 (a) contacting a cell which expresses the human hairless protein in a cell and the compound;
- (b) determining the level of expression of the human hairless protein in the cell; and
- 10 (c) comparing the level of expression of the human hairless protein determined in step (b) with the level determined in the absence of the compound, thereby identifying a compound capable of inhibiting or enhancing expression of the human hairless protein.

15 34. The method of claim 33, wherein step (a) comprises contacting a nucleic acid which expresses the human hairless protein in a cell-free expression system and the compound.

20 35. A compound, not previously known, identified by the method of claim 33 or 34.

25 36. The method of claim 33, wherein the cell is a dermal papilla cell, an epithelial cell, a follicle cell, a hair matrix cell, a hair bulb cell, a keratinocyte, a epidermal keratinocyte, a fibroblast, a cuticle cell, a medullary cell, a cortical cell, or a thymic cell.

30 37. The method of claim 33, wherein the compound is a peptide, a peptidomimetic, a nucleic acid, a polymer, or a small molecule.

38. The method of claim 33, wherein the compound is bound to a solid support.

35 39. A method for identifying a binding compound which is capable of forming a complex with a human hairless protein comprising:

- (a) contacting the human hairless protein and the compound; and

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- (b) determining the formation of a complex between the human hairless protein and the compound, thereby identifying a binding compound which is capable of forming a complex with a human hairless protein.

5

40. A compound, not previously known, identified by the method of claim 39.

10

41. A method for identifying an inhibitory compound which is capable of interfering the capacity of a human hairless protein to form a complex with the binding compound identified by the method of claim 39 comprising:

15

- (a) contacting the complex and the compound;  
(b) measuring the level of the complex; and  
(c) comparing the level of complex in the presence of the compound with the amount of the complex in the absence of the complex, a reduction in level of complex thereby identifying an inhibitory compound which is capable interfering the capacity of a human hairless protein to form a complex with the binding compound identified by the method of claim 36.

20

42. A compound, not previously known, identified by the method of claim 41.

25

43. A transgenic non-human animal comprising the nucleic acid of claim 1 or 2.

30

44. A transgenic non-human animal whose somatic and germ cells contain and express a gene encoding a mutant or wildtype human hairless protein, the genes having been introduced into the animal or an ancestor of the animal at an embryonic stage and wherein the gene may be operably linked to an inducible promoter element.

35

45. The animal of claim 43 or 44, wherein the animal is a mouse.

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46. A method for identifying whether a compound is capable of ameliorating a human hairless condition in an animal comprising:
- (a) administering the compound to the transgenic animal of claim 43 or 44, wherein the animal exhibits a human hairless condition;
  - (b) determining the level of expression of the wildtype or mutant human hairless protein in the animal; and
  - (c) comparing the level expression of the wildtype or mutant human hairless protein determined in step (b) with the level of expression determined in the animal in the absence of the compound so as to identify whether the compound is capable of ameliorating the human hairless condition in the animal.
47. A compound, not previously known, identified by the method of claim 46.
48. The method of claim 46, wherein the human hairless condition is Androgenetic Alopecia (male pattern baldness), Alopecia Areata, Alopecia Totalis, Congenital Alopecia Universalis and Congenital Alopecia and Severe T-Cell Immunodeficiency.
49. A transgenic non-human knockout animal whose cells do not express a gene encoding a mutant or wildtype human hairless protein.
50. A transgenic non-human knockout animal whose somatic and germ cells contain and do not express a gene encoding a mutant or wildtype human hairless protein, the genes having been deleted or incapacitate in the animal or an ancestor of the animal at an embryonic stage.
51. The animal of claim 49 or 50, wherein the animal is a mouse.



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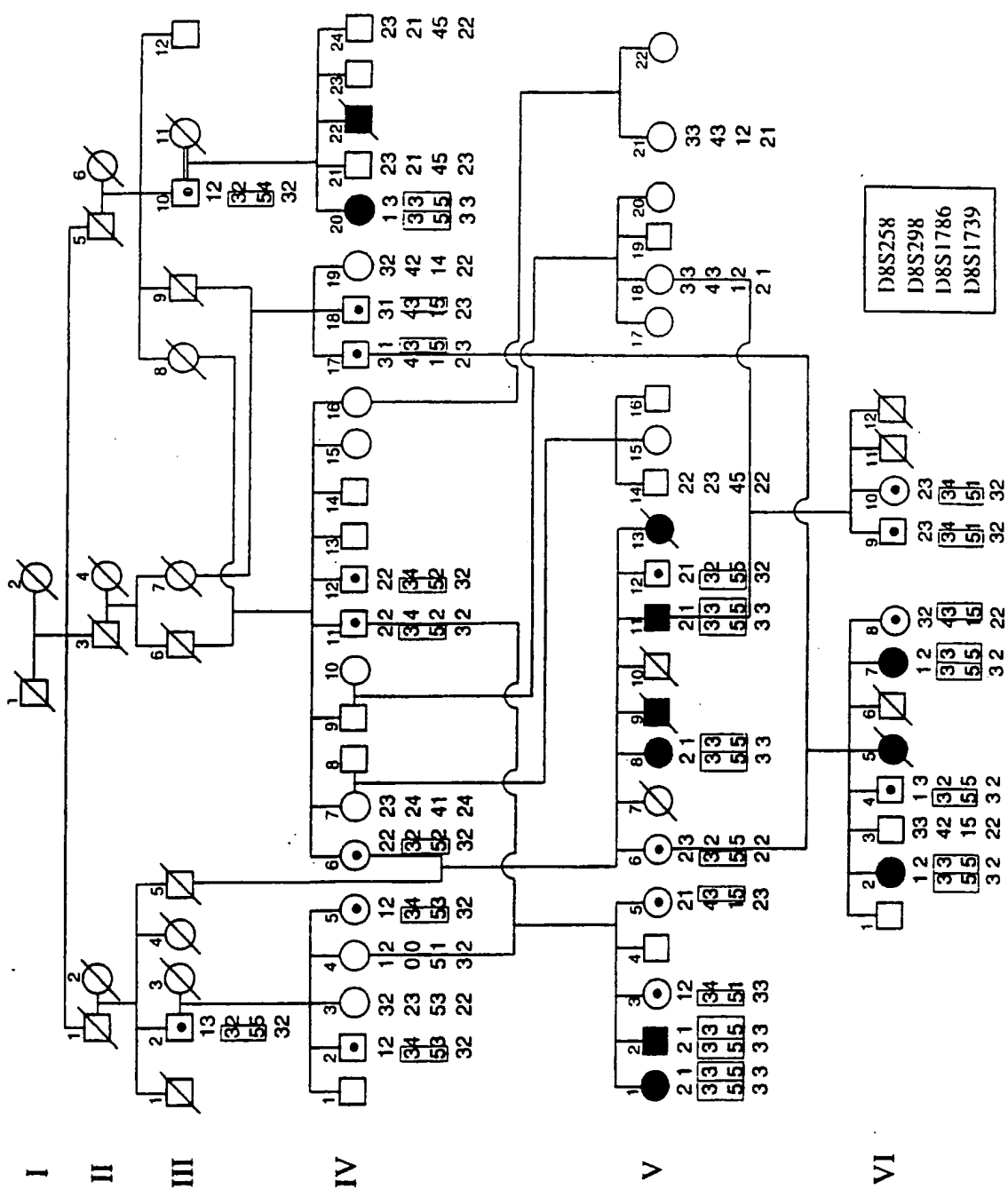
52. A method for identifying a compound capable of restoring normal phenotype to the animal of claim 49 or 50, comprising:
- 5 (a) administering the compound to the animal, wherein the animal exhibits a human hairless condition;
- 10 (b) comparing the exhibition of the condition in the animal in the presence of the compound with the exhibition of the condition in the animal in the absence of the compound so as to identify whether the compound is capable of restoring normal phenotype to the animal.
53. A compound, not previously known, identified by the method of claim 52.
- 15 54. The method of claim 52, wherein the human hairless condition is Androgenetic Alopecia (male pattern baldness), Alopecia Areata, Alopecia Totalis, Alopecia Universalis, Congenital Alopecia Universalis or Congenital Alopecia and Severe T-Cell Immunodeficiency.
- 20 55. A pharmaceutical composition which comprises a compound identified by the method of claim 33, 34, 39, 41, 46, or 48 and a pharmaceutically acceptable carrier.
- 25 56. The pharmaceutical composition of claim 52, wherein the carrier is a diluent, an aerosol, a topical carrier, an aqueous solution, a nonaqueous solution or a solid carrier.
- 30 57. A method for treating a human hairless condition in a subject comprising administering to the subject an amount of the pharmaceutical composition of claim 52, effective to treat the human hairless condition in the subject.
- 35 58. The method of claim 52, wherein the human hairless condition is Androgenetic Alopecia (male pattern baldness), Alopecia Areata, Alopecia Totalis, Alopecia

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Universalis, Congenital Alopecia Universalis or  
Congenital Alopecia and Severe T-Cell Immunodeficiency.

- 5      59. An antibody which binds specifically to the protein of  
claim 18 or 19 or portion thereof.
60. The antibody of claim 54, wherein the antibody is human.
- 10     61. The antibody of claim 54, wherein the antibody is  
monoclonal.
62. A cell producing the antibody of claim 54.
- 15     63. A method of identifying the protein of claim 21, 22 or  
24 in a sample comprising:  
      (a) contacting the sample with the antibody of claim 59  
          under conditions permissive to the formation of a  
          complex between the antibody and the protein;  
20       (b) determining the amount of complex formed; and  
      (c) comparing the amount of complex formed with the  
          amount of complex formed in the absence of the  
          sample, the presence of an increased amount of  
25       complex formed in the presence of the sample  
          indicating identification of the protein in the  
          sample.
64. A method of inhibiting hair growth in a subject,  
comprising administering to the subject an amount of the  
30     pharmaceutical composition of claim 55, effective to  
inhibit hair growth in the subject.

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FIG. 2A



FIG. 2C



FIG. 2B

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FIG. 2E

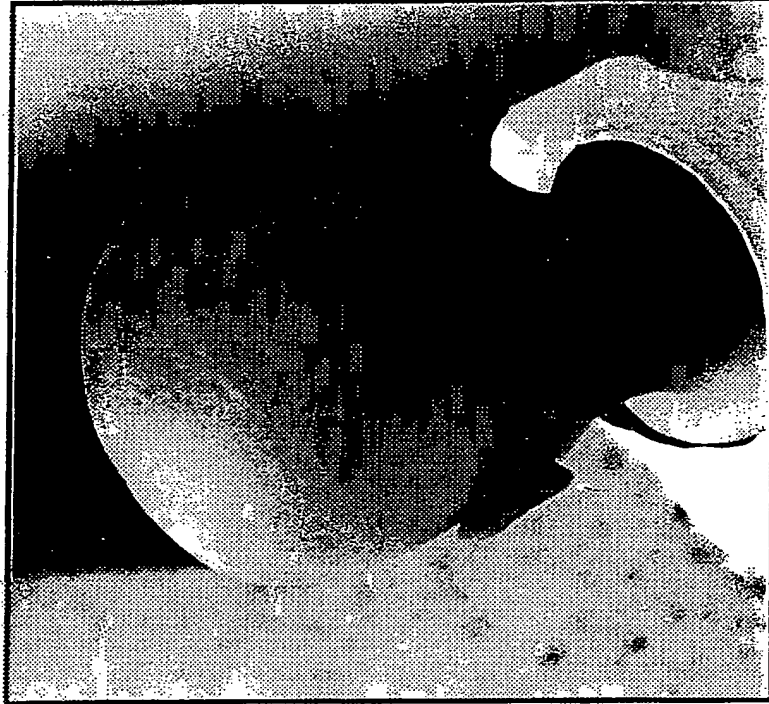
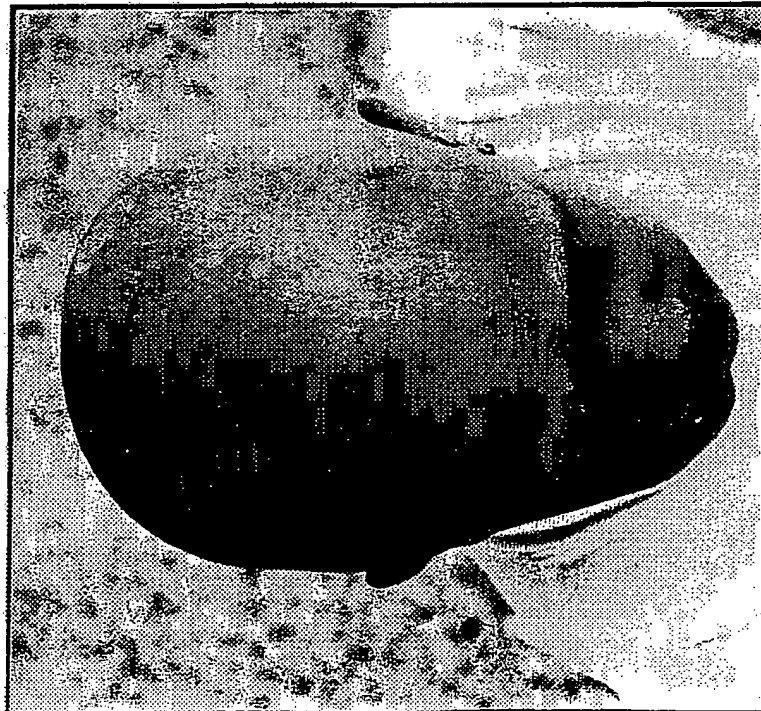


FIG. 2D

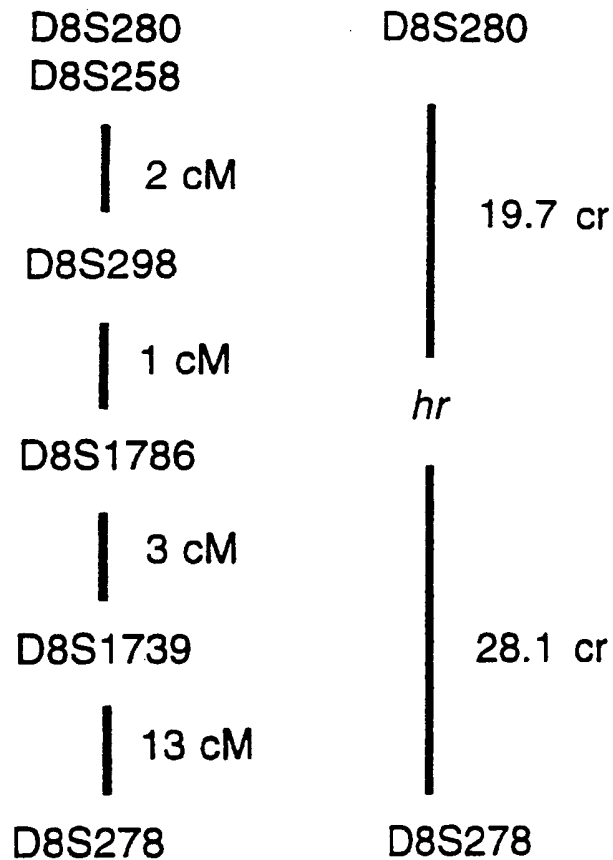


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FIG. 3A

Locus	$\theta$	Recombination fraction $\theta$						
		<u>0</u>	<u>0.01</u>	<u>0.05</u>	<u>0.1</u>	<u>0.2</u>	<u>0.3</u>	<u>0.4</u>
D8S258	$\alpha$		2.57	2.85	2.63	1.87	1.01	0.32
D8S298		6.19	6.04	5.45	4.7	3.16	1.65	0.47
D8S1786		4.92	4.83	4.43	3.92	2.87	1.79	0.76
D8S1739	$\alpha$		1.74	2.64	2.61	1.92	1	0.22

FIG. 3B



[illegible]

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FIG. 4A-2

humanhr	575	EALAWAQESQGPALVTEDESGIPHCSCSRCHHGLEFNTHWRCDECSHRLCVACGRVAGTGGAREHAFFPECSEECDEAGHAACSLILTFQVSSQALAEELSTAHQVTVKFDIRGHCHPCQA
mousehr	570	EALAWAQREGQGPANTEDESGIPHCSCSRCHHGLEFNTHWRCCHCSHRLCVACGRVAGTGGAREHAFFPECSEECDEAGHAACSLILTFQVSSQALAEELSTAHQVTVKFDIRGHCHPCQA
rathr	595	EALAWAQREGQGPANTEDESGIPHCSCSRCHHGLEFNTHWRCCHCSHRLCVACGRVAGTGGAREHAFFPECSEECDEAGHAACSLILTFQVSSQALAEELSTAHQVTVKFDIRGHCHPCQA
humanhr	695	DARVWAPGLRGGQKESTIKETPDSDAEPAEDRAGRPLPCPSLCELLASTAVKLCGHERIHMAFAPVTPALPSDDRTNILDSTIIAQVVERKIQEKAL
mousehr	690	DARVWAPGLRGGQKESTIKETPDSDAEPAEDRAGRPLPCPSLCELLASTAVKLCGHERIHMAFAPVTPALPSDDRTNILDSTIIAQVVERKIQEKAL
rathr	715	DARVWAPGLRGGQKESTIKETPDSDAEPAEDRAGRPLPCPSLCELLASTAVKLCGHERIHMAFAPVTPALPSDDRTNILDSTIIAQVVERKIQEKAL
humanhr	815	3PGLRAGEGLRKGLGLPLSPVRPRLEPPGALLMLQEPQPCPEHGFHLFQEHWRQGPVLVSGIGRTIQCNLWCTEALCALGGQVQALSPGLGPPQFSSLSSTTFWEGFSWPELRPKSDEGS
mousehr	810	3PGLRAGEGLRKGLGLPLSPVRPRLEPPGALLMLQEPQPCPEHGFHLFQEHWRQGPVLVSGIGRTIQCNLWCTEALCALGGQVQALSPGLGPPQFSSLSSTTFWEGFSWPELRPKSDEGS
rathr	835	3PGLRAGEGLRKGLGLPLSPVRPRLEPPGALLMLQEPQPCPEHGFHLFQEHWRQGPVLVSGIGRTIQCNLWCTEALCALGGQVQALSPGLGPPQFSSLSSTTFWEGFSWPELRPKSDEGS
humanhr	935	VLLLFHFLGDEETSRVENLANSPLPEYCAHGGKLNLA SYLPLGLALHPLEQLMAAYGVSTSRCHLGTKNLCVEVNDLHSILVHADTTPAHPRAQKDFLSGLDGEGLWSPGSCVSTV
mousehr	928	VLLLFHFLGDKTASRVNLAASSLPLPEYCAHGGKLNLA SYLPLGLTLHPLEQLMAAYGVNISHFGHLGTKNLCVEVSDLSILVHAEAOQLPPVYRAQKDFLSGLDGEGLWSPGSCVSTV
rathr	953	VLLLFHFLGDKTBSRVENLAASSLPLPEYCAHGGKLNLA SYLPLGLTLHPLEQLMAAYGVNISHFGHLGTKNLCVEVSDLSILVHAEAOQLPPVYRAQKDFLSGLDGEGLWSPGSCVSTV
humanhr	1055	HVFRQAQAOIRIRRELQWCPAGAGALEPGAPGSCYLDAGLRRLREEMGVSCWTLLOAPGEAVLVPAGAPHQVQGLVSTISVTQHFLSPETSALSQAQLCHQGPSPLPPECCHLLYAQMDRAV
mousehr	1048	HVFRQAQAOIRIRRELQWCPAGAGALEPGAPGSCYLDAGLRRLREEMGVSCWTLLOAPGEAVLVPAGAPHQVQGLVSTISVTQHFLSPETSALSQAQLHQQGASLPPDHRRLYAQMDRAV
rathr	1073	HVFRQAQAOIRIRRELQWCPAGAGALEPGAPGSCYLDAGLRRLREEMGVSCWTLLOAPGEAVLVPAGAPHQVQGLVSTISVTQHFLSPETSALSQAQLCHQGPSPLPPECCHLLYAQMDRAV
humanhr	1175	EQAVKVAVGTLQEAKE
mousehr	1168	EQAVKVAVGTLQEAKE
rathr	1193	EQAVKVAVGTLQEAKE



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FIG. 4B

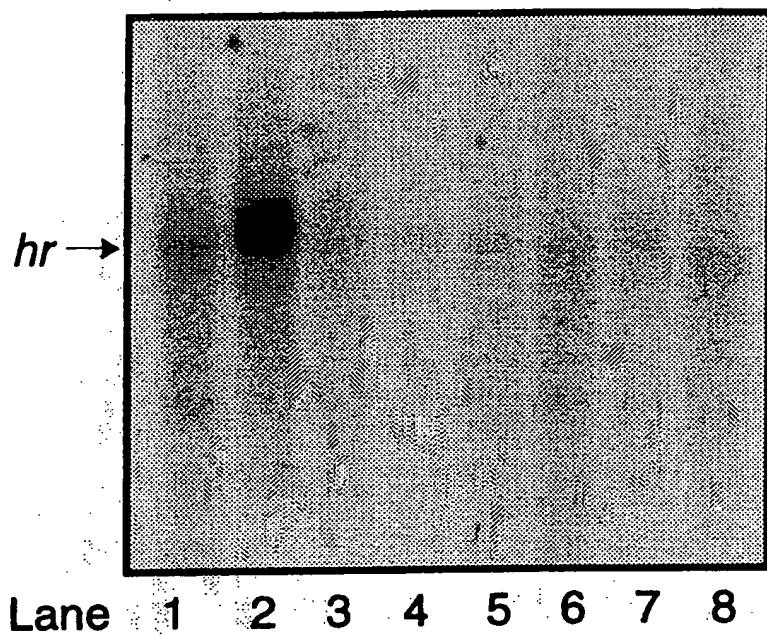


FIG. 4C



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FIG. 5A

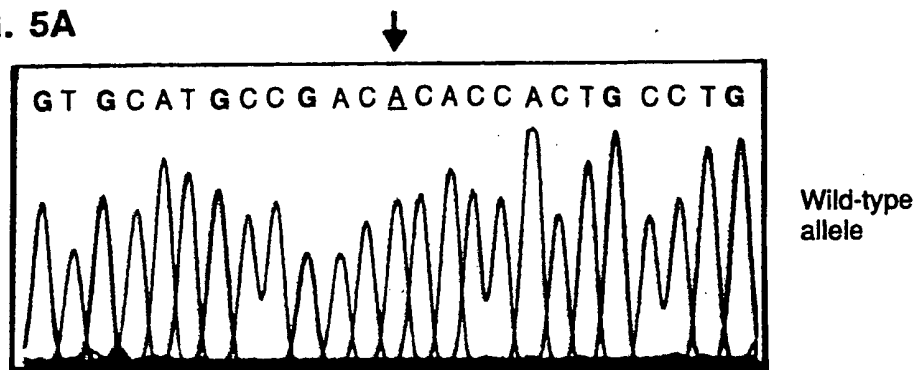


FIG. 5B

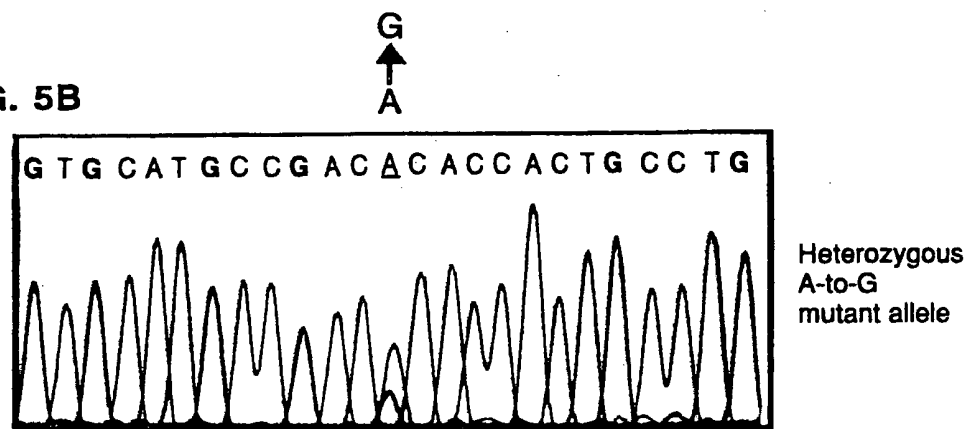
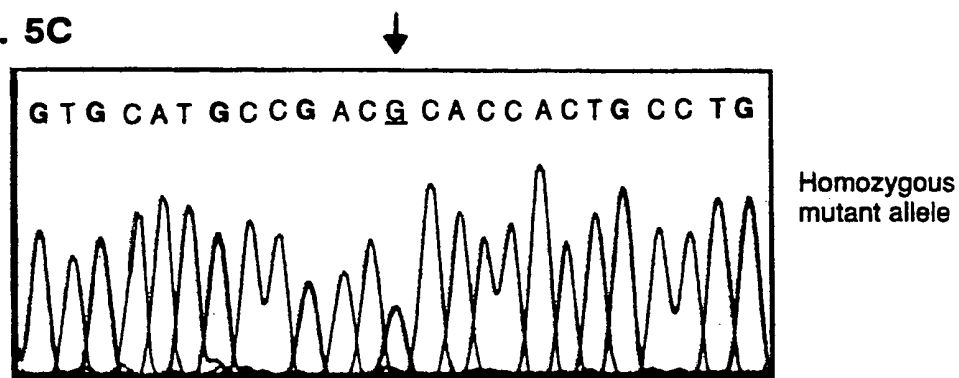


FIG. 5C



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FIG. 6A

1/1  
ATG GAG AGT ACG CCC AGC TTC CTG AAG GGC ACC CCA ACC TGG GAG AAG ACG GCC CCA GAG  
M E S T P S F L K G T P T W E K T A P E  
61/21  
AAC GGC ATC GTG AGA CAG GAG CCC GGC AGC CCG CCT CGA GAT GGA CTG CAC CAT GGG CCG  
N G I V R Q E P G S P P R D G L H H G P  
121/41  
CTG TGC CTG GGA GAG CCT GCT CCC TTT TGG AGG GGC GTC CTG AGC ACC CCA GAC TCC TGG  
L C L G E P A P F W R G V L S T P D S W  
181/61  
CTT CCC CCT GGC TTC CCC CAG GGC CCC AAG GAC ATG CTC CCA CTT GTG GAG GGC GAG GGC  
L P P G F P Q G P K D M L P L V E G E G  
241/81  
CCC CAG AAT GGG GAG AGG AAG GTC AAC TGG CTG GGC AGC AAA GAG GGA CTG CGC TGG AAG  
P Q N G E R K V N W L G S K E G L R W K  
301/101  
GAG GCC ATG CTT ACC CAT CCG CTG GCA TTC TGC GGG CCA GCG TGC CCA CCT CGC TGT GGC  
E A M L T H P L A F C G P A C P P R C G  
361/121  
CCC CTG ATG CCT GAG CAT AGT GGT GGC CAT CTC AAG AGT GAC CCT GTG GCC TTC CGG CCC  
P L M P E H S G G H L K S D P V A F R P  
421/141  
TGG CAC TGC CCT TTC CTT CTG GAG ACC AAG ATC CTG GAG CGA GCT CCC TTC TGG GTG CCC  
W H C P F L L E T K I L E R A P F W V P  
481/161  
ACC TGC TTG CCA CCC TAC CTA GTG TCT GGC CTG CCC CCA GAG CAT CCA TGT GAC TGG CCC  
T C L P P Y L V S G L P P E H P C D W P  
541/181  
CTG ACC CCG CAC CCC TGG GTA TAC TCC GGG GGC CAG CCC AAA GTG CCC TCT GCC TTC AGC  
L T P H P W V Y S G G Q P K V P S A F S  
601/201  
TTA GGC AGC AAG GGC TTT TAC TAC AAG GAT CCG AGC ATT CCC AGG TTG GCA AAG GAG CCC  
L G S K G F Y Y K D P S I P R L A K E P  
661/221  
TTG GCA GCT GCG GAA CCT GGG TTG TTT GGC TTA AAC TCT GGT GGG CAC CTG CAG AGA GCC  
L A A A E P G L F G L N S G G H L Q R A  
721/241  
GGG GAG GCC GAA CGC CCT TCA CTG CAC CAG AGG GAT GGA GAG ATG GGA GCT GGC CGG CAG  
G E A E R P S L H Q R D G E M G A G R Q  
781/261  
CAG AAT CCT TGC CCG CTC TTC CTG GGG CAG CCA GAC ACT GTG CCC TGG ACC TCC TGG CCC  
Q N P C P L F L G Q P D T V P W T S W P  
841/281  
GCT TGT CCC CCA GGC CTT GTT CAT ACT CTT GGC AAC GTC TGG GCT GGG CCA GGC GAT GGC  
A C P P G L V H T L G N V W A G P G D G  
901/301  
AAC CTT GGG TAC CAG CTG GGG CCA CCA GCA ACA CCA AGG TGC CCC TCT CCT GAG CCG CCT  
N L G Y Q L G P P A T P R C P S P E P P  
961/321  
GTC ACC CAG CGG GGC TGC TGT TCA TCC TAC CCA CCC ACT AAA GGT GGG GAT CTT GGC CCT  
V T Q R G C C S S Y P P T K G G D L G P  
1021/341  
TGT GGG AAG TGC CAG GAG GGC CTG GAG GGG GGT GCC AGT GGA GCC AGC GAA CCC AGC GAG  
C G K C Q E G L E G G A S G A S E P S E  
1081/361  
GAA GTG AAC AAG GCC TCT GGC CCC AGG GCC TGT CCC CCC AGC CAC CAC ACC AAG CTG AAG  
E V N K A S G P R A C P P S H H T K L K  
1141/381  
AAG ACA TGG CTC ACA CGG CAC TCG GAG CAG TTT GAA TGT CCA CGC GGC TGC CCT GAG GTC  
K T W L T R H S E Q F E C P R G C P E V  
1201/401  
GAG GAG AGG CCG GTT GCT CGG CTC CGG GCC CTC AAA AGG GCA GGC AGC CCC GAG GTC CAG  
E E R P V A R L R A L K R A G S P E V Q

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FIG. 6B

1261/421  
 GGA GCA ATG GGC AGT CCA GCC CCC AAG CGG  
 G A M G S P A P K R  
 1321/441  
 CAG GGG GCT GGG GGT TTG CAG GAG GTG CGG  
 Q G A G G L Q E V R D T S I G N K D V D  
 1381/461  
 TCG GGA CAG CAT GAT GAG CAG AAA GGA CCC  
 S G Q H D E Q K G P Q D G Q A S L Q D P  
 1441/481  
 GGA CTT CAG GAC ATA CCA TGC CTG GCT CTC  
 G L Q D I P C L A L P A K L A Q C Q S C  
 1501/501  
 GCC CAG GCA GCT GGA GAG GGA GGA GGG CAC  
 A Q A A G E G G G H A C H S Q Q V R R S  
 1561/521  
 CCT CTG GGA GGG GAG CTG CAG CAG GAG GAA  
 P L G G E L Q Q E E D T A T N S S S E E  
 1621/541  
 GGC CCA GGG TCC GGC CCT GAC AGC CGG CTC  
 G P G S G P D S R L S T G L A K H L L S  
 1681/561  
 GGT TTG GGG GAC CGA CTG TGC CGC CTG CTG  
 G L G D R L C R L L R R E R E A L A W A  
 1741/581  
 CAA CGG GAA AGC CAA GGG CCA GCC GTG ACA  
 Q R E S Q G P A V T E D S P G I P R C C  
 1801/601  
 AGC CGT TGC CAC CAT GGA CTC TTC AAC ACC  
 S R C H H G L F N T H W R C P R C S H R  
 1861/621  
 CTG TGT GTG GCC TGT GGT CGT GTG GCA GGC  
 L C V A C G R V A G T G R A R E K A G F  
 1921/641  
 CAG GAG CAG TCC GCG GAG GAG TGC ACG CAG  
 Q E Q S A E E C T Q E A G H A A C S L M  
 1981/661  
 CTG ACC CAG TTT GTC TCC AGC CAG GCT TTG  
 L T Q F V S S Q A L A E L S T A M H Q V  
 2041/681  
 TGG GTC AAG TTT GAT ATC CGG GGG CAC TGC  
 W V K F D I R G H C P C Q A D A R V W A  
 2101/701  
 CCC GGG GAT GCA GGC CAG CAG AAG GAA TCA  
 P G D A G Q Q K E S T Q K T P P T P Q P  
 2161/721  
 TCC TGC AAT GGC GAC ACC CAC AGG ACC AAG  
 S C N G D T H R T K S I K E E T P D S A  
 2221/741  
 GAG ACC CCA GCA GAG GAC CGT GCT GGC CGA  
 E T P A E D R A G R G P L P C P S L C E  
 2281/761  
 CTG CTG GCT TCT ACC GCG GTC AAA CTC TGC  
 L L A S T A V K L C L G H E R I H M A F  
 2341/781  
 GCC CCC GTC ACT CCG GCC CTG CCC AGT GAT  
 A P V T P A L P S D G A C G C A T C C A A C A T C T G A C A G C A T T  
 2401/801  
 ATC GCA CAG GTG GTG GAA CGG AAG ATC CAG  
 I A Q V V E R K I Q E K A L G P G L R A  
 2461/821  
 GGC CCG GGT CTG CCG AAG GGC CTG GGC CTG  
 G P G L R K G L G L P L S P V R P R L P  
 2521/841  
 CCC CCA GGG GCT TTG CTG TGG CTG CAG GAG  
 P P G A L L W L Q E P Q P C P R R G F H  
 2581/861  
 CTC TTC CAG GAG CAC TGG AGG CAG GGC CAG  
 L F Q E H W R Q G Q P V L V S G I Q R T

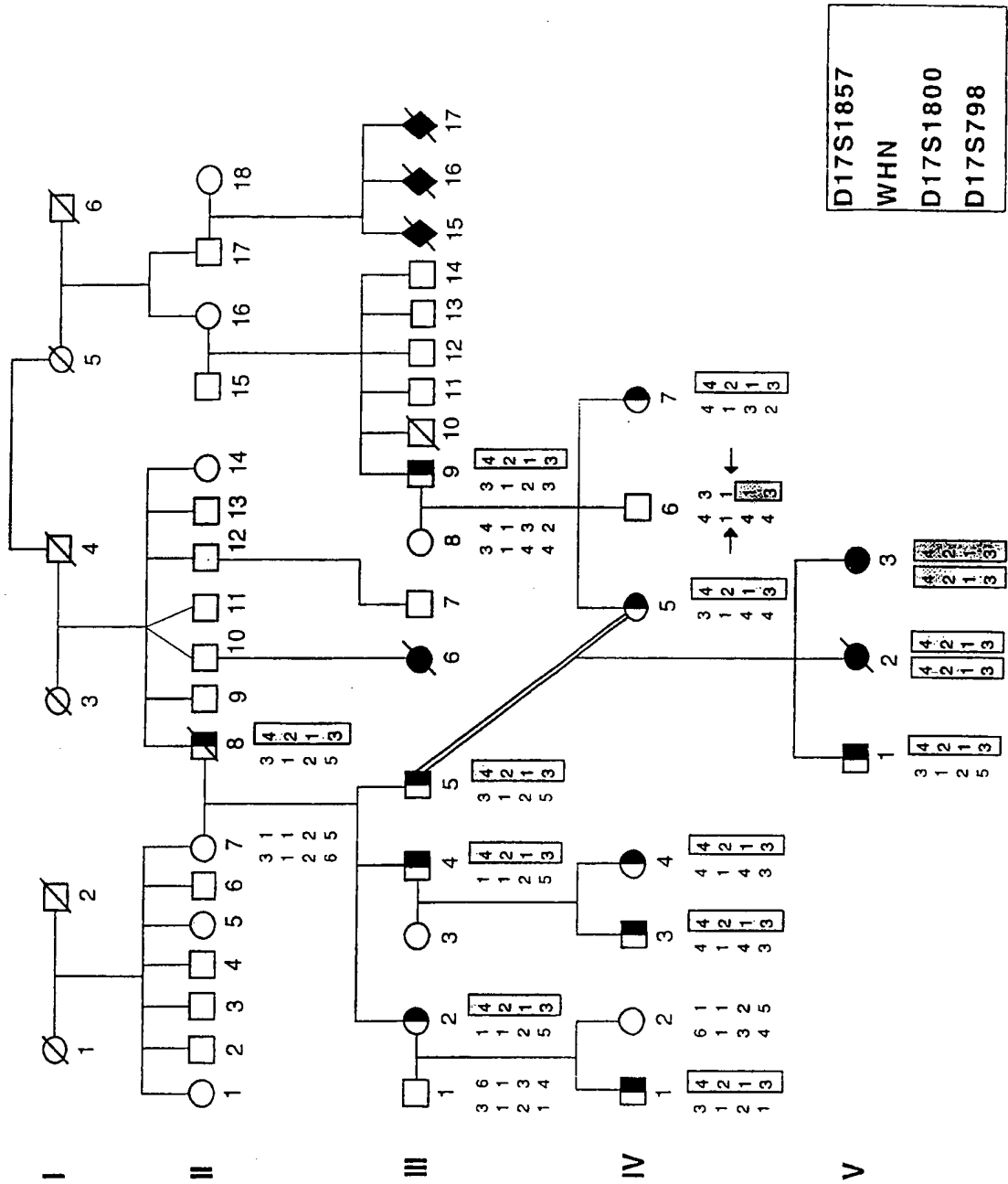
11/21

FIG. 6C

2641/881  
 TTG CAG GGC AAC CTG TGG GGG ACA GAA GCT CTT GGG GCA CTT GGA GGC CAG GTG CAG GCG  
 L Q G N L W G T E A L G A L G G Q V Q A  
 2701/901  
 CTG AGC CCC CTC GGA CCT CCC CAG CCC AGC AGC CTG GGC AGC ACA ACA TTC TGG GAG GGC  
 L S P L G P P Q P S S L G S T T F W E G  
 2761/921  
 TTC TCC TGG CCT GAG CTT CGC CCA AAG TCA GAG GAG GGC TCT GTC CTC CTG CTG CAC CGA  
 F S W P E L R P K S D E G S V L L L H R  
 2821/941  
 GCT TTG GGG GAT GAG GAC ACC AGC AGG GTG GAG AAC CTA GCT GCC AGT CTG CCA CTT CCG  
 A L G D E D T S R V E N L A A S L P L P  
 2881/961  
 GAG TAC TGC GCC CTC CAT GGA AAA CTC AAC CTG GCT TCC TAC CTC CCA CCG GGC CTT GCC  
 E Y C A L H G K L N L A S Y L P P G L A  
 2941/981  
 CTG CGT CCA CTG GAG CCC CAG CTC TGG GCA GCC TAT GGT GTG AGC CCG CAC CGG GGA CAC  
 L R P L E P Q L W A A Y G V S P H R G H  
 3001/1001  
 CTG GGG ACC AAG AAC CTC TGT GTG GAG GTG GCC GAC CTG GTC AGC ATC CTG GTG CAT GCC  
 L G T K N L C V E V A D L V S I L V H A  
 3061/1021  
 GAC ACA CCA CTG CCT GCC TGG CAC CGG GCA CAG AAA GAC TTC CTT TCA GGC CTG GAC GGG  
 D T P L P A W H R A Q K D F L S G L D G  
 3121/1041  
 GAG GGG CTC TGG TCT CCG GGC AGC CAG GTC AGC ACT GTG TGG CAC GTG TTC CGG GCA CAG  
 E G L W S P G S Q V S T V W H V F R A Q  
 3181/1061  
 GAC GCC CAG CGC ATC CGC CGC TTT CTC CAG ATG GTG TGC CCG GCC GGG GCA GGC GCC CTG  
 D A Q R I R R F L Q M V C P A G A G A L  
 3241/1081  
 GAG CCT GGC GCC CCA GGC AGC TGC TAC CTG GAT GCA GGG CTG CGG CGG CGC CTG CGG GAG  
 E P G A P G S C Y L D A G L R R R L R E  
 3301/1101  
 GAG TGG GGC GTG AGC TGC TGG ACC CTG CTC CAG GCC CCC GGA GAG GCC GTG CTG GTG CCT  
 E W G V S C W T L L Q A P G E A V L V P  
 3361/1121  
 GCA GGG GCT CCC CAC CAG GTG CAG GGC CTG GTG AGC ACA GTC AGC GTC ACT CAG CAC TTC  
 A G A P H Q V Q G L V S T V S V T Q H F  
 3421/1141  
 CTC TCC CCT GAG ACC TCT GCC CTC TCT GCT CAG CTC TGC CAC CAG GGA CCC AGC CTT CCC  
 L S P E T S A L S A Q L C H Q G P S L P  
 3481/1161  
 CCT GAC TGC CAC CTG CTT TAT GCC CAG ATG GAC TGG GCT GTG TTC CAA GCA GTG AAG GTG  
 P D C H L L Y A Q M D W A V F Q A V K V  
 3541/1181  
 GCC GTG GGG ACA TTA CAG GAG GCC AAA  
 A V G T L Q E A K

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FIG. 7A



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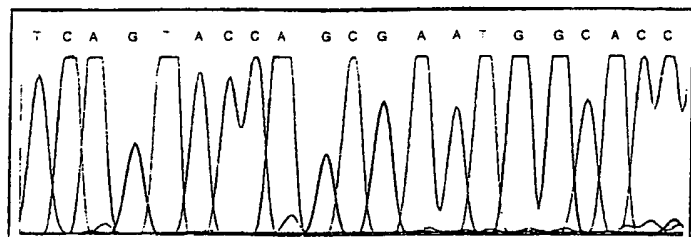
FIG. 7B

Recombination fraction  $\theta$ 

Locus	<u>0.00</u>	<u>0.05</u>	<u>0.1</u>	<u>0.2</u>	<u>0.3</u>	<u>0.4</u>
D17S1857	1.02	0.91	0.80	0.58	0.37	0.17
D17S1800	1.32	1.21	1.1	0.86	0.60	0.32
D17S798	1.32	1.21	1.1	0.86	0.60	0.32

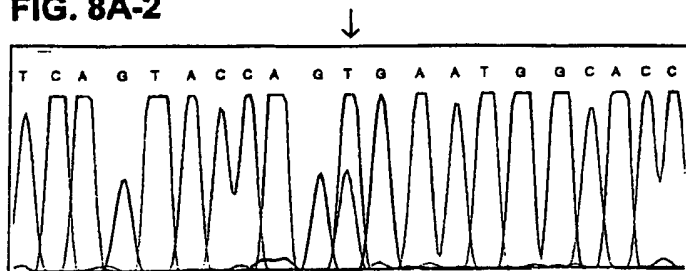
14/21

FIG. 8A-1



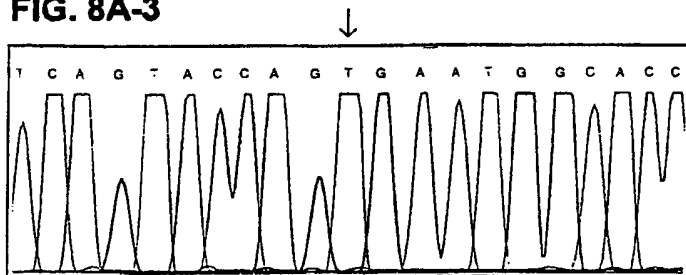
WHN  
wild-type allele

FIG. 8A-2



WHN  
R255X heterozygous  
mutant allele

FIG. 8A-3



WHN  
R255X homozygous  
mutant allele



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FIG. 8B

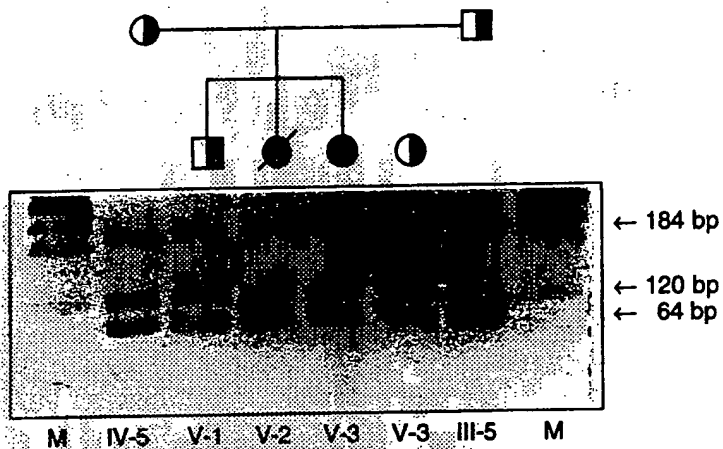
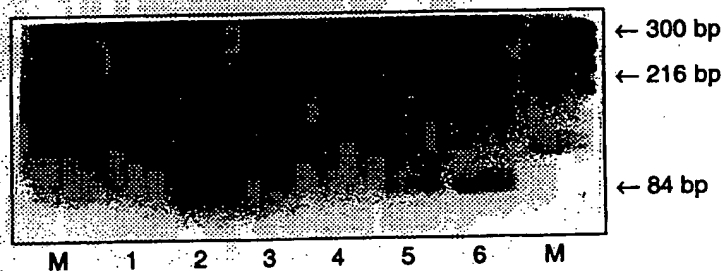


FIG. 8C



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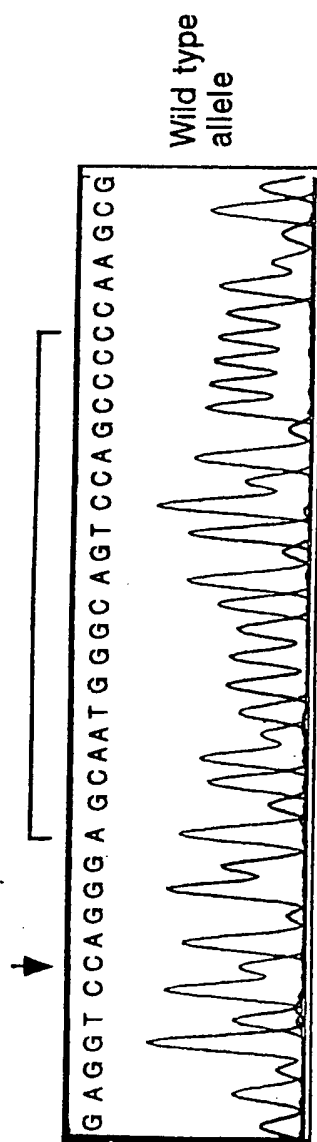


FIG. 8D-1

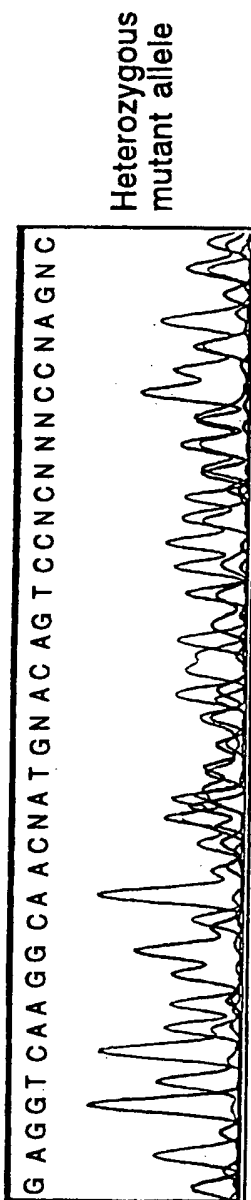


FIG. 8D-2

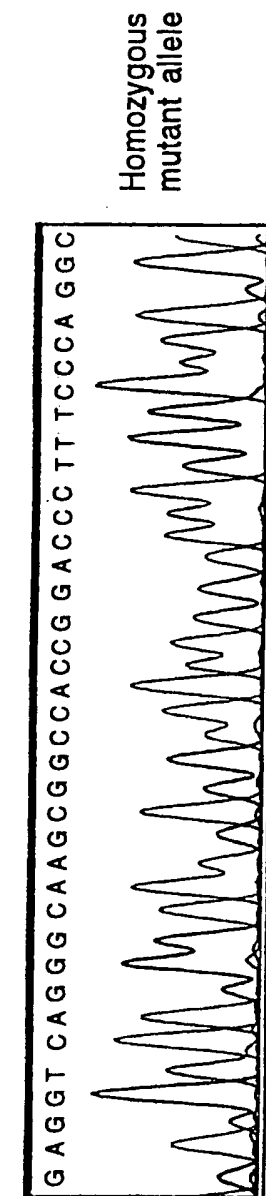


FIG. 8D-3

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FIG. 9A

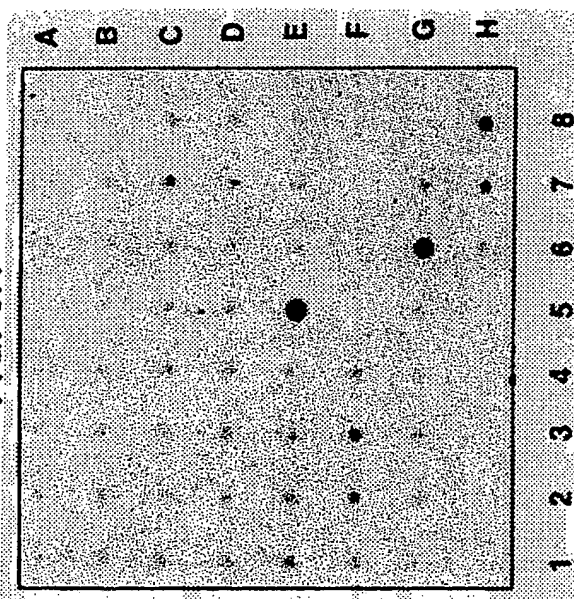


FIG. 9B

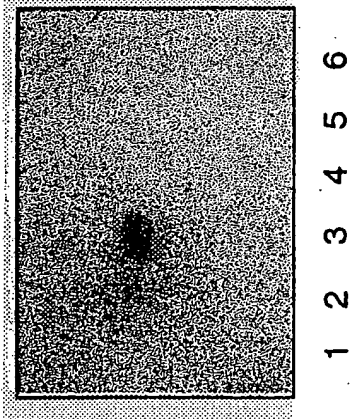


FIG. 9C

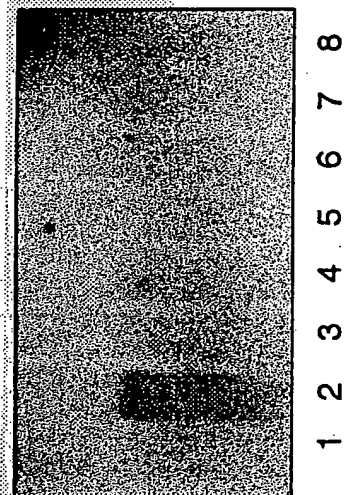
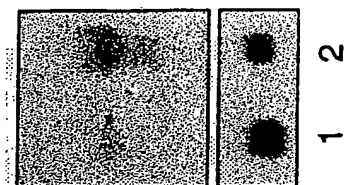


FIG. 9D



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FIG. 10B

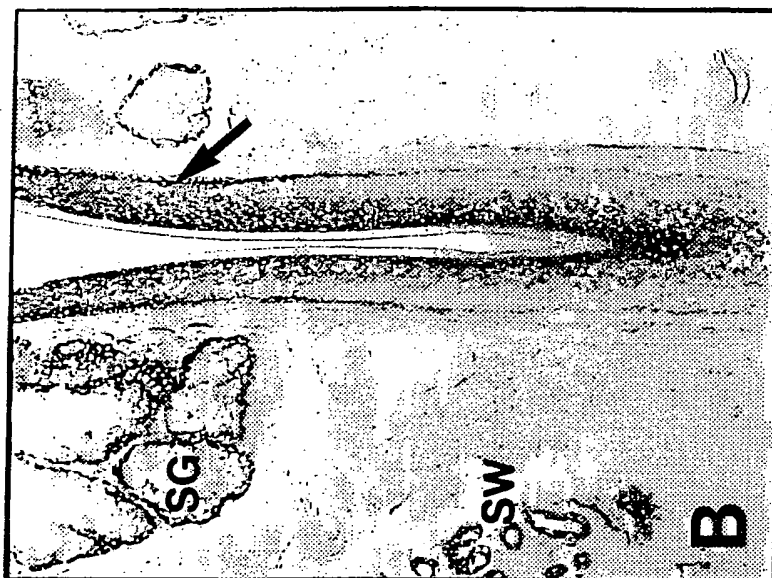


FIG. 10A



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FIG. 10D

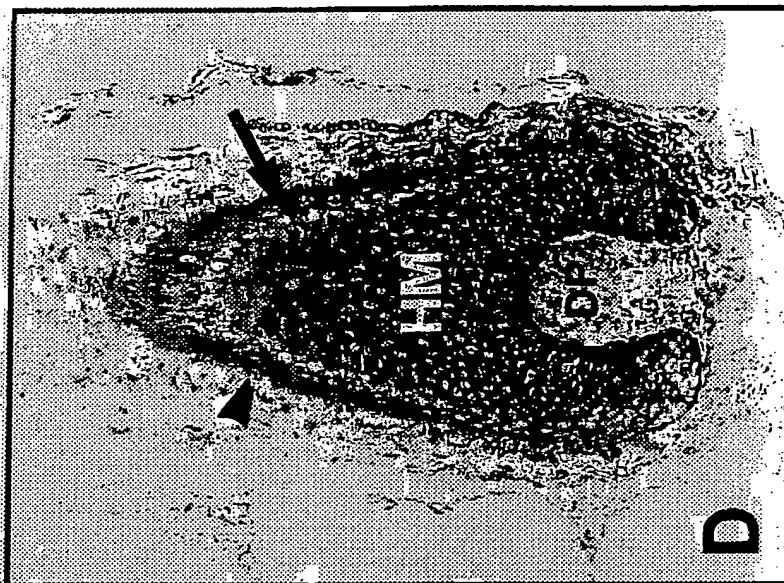
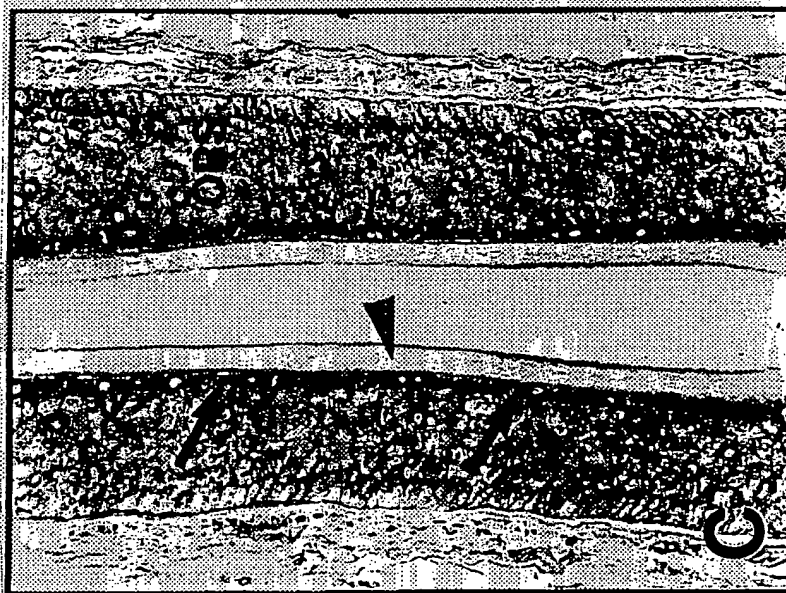


FIG. 10C



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FIG. 11

Summary of hairless gene mutations

<u>rhino mouse</u>	<u>mutation</u>	<u>consequence</u>	<u>phenotype</u>
rh-8J	K512X	nonsense/exon 4	severe
rh-farm mouse	R467X	nonsense/exon 4	severe
rh-J	3 bp deletion	exon skip/exon 5	severe
rh-J9	insertion intron 5	?	severe
<u>hairless mouse</u>			
HRS/J	insertion intron 6	aberrant splicing?	moderate
<u>human atrichia</u>			
Pakistani Family	T1022A	missense/exon 15	mild
Irish Traveller Family	R620Q	missense/exon 6	mild
Arab Families-six	2147delC	frameshift exon 9/PTC	mild/moderate
Arab Palestinian Family	1256delC, 1261del21	frameshift/PTC	mild
Japanese Family	R613X	nonsense/exon 6	mild
Polish Family	C622G	missense/exon 6	mild/moderate

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FIG. 12A

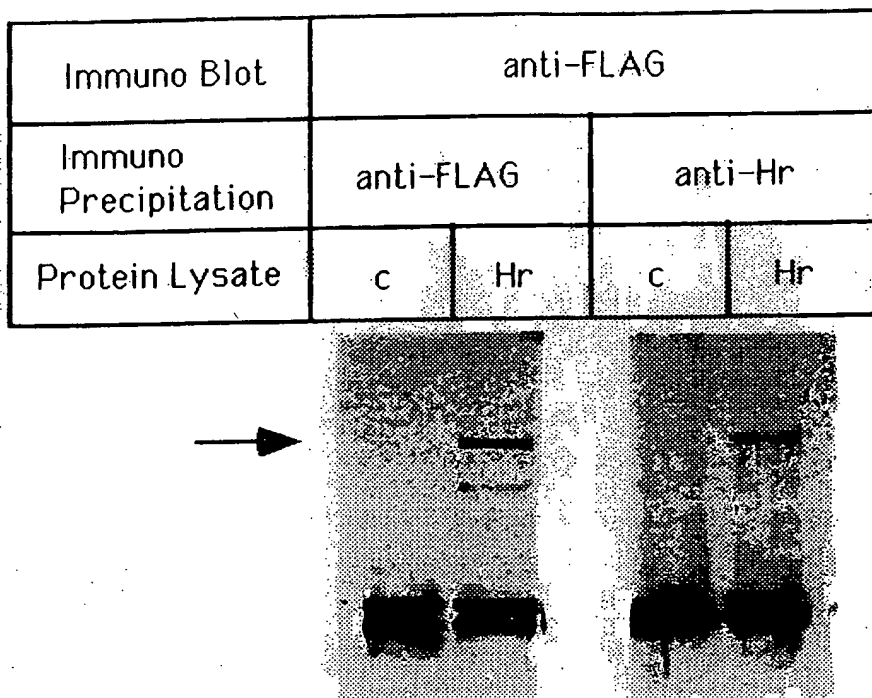
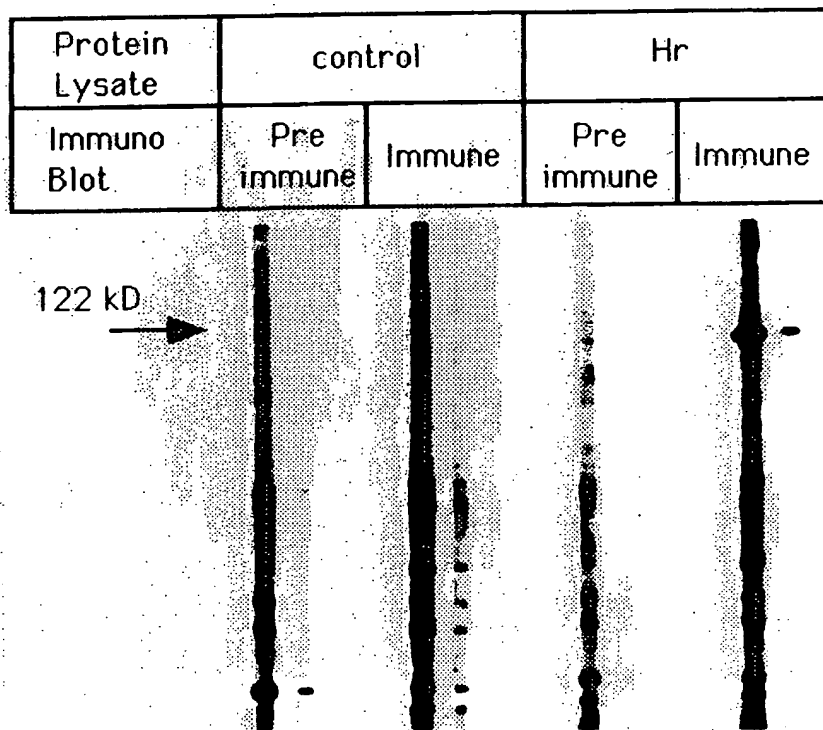


FIG. 12B



## SEQUENCE LISTING

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<120> Human Hairless Gene, Protein and Uses Thereof

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<140> Not Yet known

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<170> PatentIn Ver. 2.0

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&lt;212&gt; PRT

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Pro Gln Asn Gly Glu Arg Lys Val Asn Trp Leu Gly Ser Lys Glu Gly  
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Leu Arg Trp Lys Glu Ala Met Leu Thr His Pro Leu Ala Phe Cys Gly  
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Pro Ala Cys Pro Pro Arg Cys Gly Pro Leu Met Pro Glu His Ser Gly  
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Gly His Leu Lys Ser Asp Pro Val Ala Phe Arg Pro Trp His Cys Pro  
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Phe Leu Leu Glu Thr Lys Ile Leu Glu Arg Ala Pro Phe Trp Val Pro  
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Cys Asp Trp Pro Leu Thr Pro His Pro Trp Val Tyr Ser Gly Gly Gln  
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Pro Lys Val Pro Ser Ala Phe Ser Leu Gly Ser Lys Gly Phe Tyr Tyr  
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Lys Asp Pro Ser Ile Pro Arg Leu Ala Lys Glu Pro Leu Ala Ala Ala  
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Glu Pro Gly Leu Phe Gly Leu Asn Ser Gly Gly His Leu Gln Arg Ala  
 225 230 235 240

Gly Glu Ala Glu Arg Pro Ser Leu His Gln Arg Asp Gly Glu Met Gly  
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Thr Val Pro Trp Thr Ser Trp Pro Ala Cys Pro Pro Gly Leu Val His  
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Asp Leu Gly Pro Cys Gly Lys Cys Gln Glu Gly Leu Glu Gly Gly Ala  
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Arg Ala Cys Pro Pro Ser His His Thr Lys Leu Lys Lys Thr Trp Leu  
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Glu Glu Arg Pro Val Ala Arg Leu Arg Ala Leu Lys Arg Ala Gly Ser  
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Pro Glu Val Gln Gly Ala Met Gly Ser Pro Ala Pro Lys Arg Pro Pro  
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Asp Pro Phe Pro Gly Thr Ala Glu Gln Gly Ala Gly Gly Leu Gln Glu  
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Gly Pro Asp Ser Arg Leu Ser Thr Gly Leu Ala Lys His Leu Leu Ser  
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 690 695 700  
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 Ser Cys Asn Gly Asp Thr His Arg Thr Lys Ser Ile Lys Glu Glu Thr  
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 740 745 750  
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Gly Leu Arg Ala Gly Pro Gly Leu Arg Lys Gly Leu Gly Leu Pro Leu  
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Ser Pro Val Arg Pro Arg Leu Pro Pro Pro Gly Ala Leu Leu Trp Leu  
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Gly Ser Thr Thr Phe Trp Glu Gly Phe Ser Trp Pro Glu Leu Arg Pro  
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Lys Ser Asp Glu Gly Ser Val Leu Leu Leu His Arg Ala Leu Gly Asp  
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Glu Asp Thr Ser Arg Val Glu Asn Leu Ala Ala Ser Leu Pro Leu Pro  
 945 950 955 960

Glu Tyr Cys Ala Leu His Gly Lys Leu Asn Leu Ala Ser Tyr Leu Pro  
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Pro Gly Leu Ala Leu Arg Pro Leu Glu Pro Gln Leu Trp Ala Ala Tyr  
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Gly Val Ser Pro His Arg Gly His Leu Gly Thr Lys Asn Leu Cys Val  
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Glu Val Ala Asp Leu Val Ser Ile Leu Val His Ala Asp Thr Pro Leu  
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Pro Ala Trp His Arg Ala Gln Lys Asp Phe Leu Ser Gly Leu Asp Gly  
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Glu Gly Leu Trp Ser Pro Gly Ser Gln Val Ser Thr Val Trp His Val  
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Met Leu Ala His Pro Leu Ala Phe Cys Gly Pro Ala Cys Pro Pro Arg	130		135		140
Tyr Gly Pro Leu Ile Pro Glu His Ser Ser Gly His Pro Lys Ser Asp	145		150		155
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Pro Val Ala Phe Arg Pro Leu His Cys Pro Phe Leu Leu Glu Thr Lys	165		170		175
Ile Leu Glu Arg Ala Pro Phe Trp Val Pro Thr Cys Leu Pro Pro Tyr	180		185		190
Leu Met Ser Ser Leu Pro Pro Glu Arg Ser Tyr Asp Trp Pro Leu Ala	195		200		205
Pro Ser Pro Trp Val Tyr Ser Gly Ser Gln Pro Lys Val Pro Ser Ala	210		215		220
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					240
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Ser Leu His Gln Arg Asp Gly Glu Thr Gly Ala Gly Arg Gln Gln Asn	275		280		285
Leu Cys Pro Val Phe Leu Gly Tyr Pro Asp Thr Val Pro Arg Thr Pro	290		295		300
Trp Pro Ser Cys Pro Pro Gly Leu Val His Thr Leu Gly Asn Val Trp	305		310		315
					320
Ala Gly Pro Gly Ser Asn Ser Phe Gly Tyr Gln Leu Gly Pro Pro Val	325		330		335
Thr Pro Arg Cys Pro Ser Pro Gly Pro Pro Thr Pro Pro Gly Gly Cys					

9



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His Gly Leu Phe Asn Thr His Trp Arg Cys Ser His Cys Ser His Arg		
625	630	635 640
Leu Cys Val Ala Cys Gly Arg Ile Ala Gly Ala Gly Lys Asn Arg Glu		
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Lys Thr Gly Ser Arg Glu Gln Arg Thr Asp Asp Cys Ala Gln Glu Ala		
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Gly His Ala Ala Cys Ser Leu Ile Leu Thr Gln Phe Val Ser Ser Gln		
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Gly Arg Ser Pro Leu Pro Cys Pro Ser Leu Cys Glu Leu Leu Ala Ser		
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Leu Asp Ser Ile Ile Ala Gln Val Val Glu Arg Lys Ile Gln Glu Lys		
820	825	830
Ala Leu Gly Pro Gly Leu Arg Ala Gly Ser Gly Leu Arg Lys Gly Leu		
835	840	845
Ser Leu Pro Leu Ser Pro Val Arg Thr Gln Leu Ser Pro Pro Gly Ala		

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Ser Cys Tyr Leu Asp Ser Gly Leu Arg Arg Arg Leu Arg Glu Glu Trp		

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 Ser Val Thr Gln His Phe Leu Ser Pro Glu Thr Ser Ala Leu Ser Ala  
                          1155                      1160                      1165  
 Gln Leu Cys His Gln Gly Ala Ser Leu Pro Pro Asp His Arg Met Leu  
                          1170                      1175                      1180  
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 Thr Leu Gln Glu Ala Lys  
                          1205

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&lt;211&gt; 1189

&lt;212&gt; PRT

&lt;213&gt; Homosapien

&lt;400&gt; 4

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22

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/02128

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C12N 15/11; C07K 14/00; A61K 7/06, 39/395; A01K 67/00

US CL : 536/23.1; 530/350; 424/70.1, 130.1 800/8

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 530/350; 424/70.1, 130.1 800/8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: human hairless protein, alopecia, gene

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	AHMAD et al. Alopecia universalis associated with a mutation in the human hairless gene. Science. 30 January 1998, Vol 279, pages 720-724, abstract only.	1-44,49-54, 56-58
A,P	CICHON et al. Cloning, genomic organization, alternative transcripts and mutational analysis of the gene responsible for autosomal recessive universal congenital alopecia. Human Molecular Genetics. October 1998, Vol. 7, No. 11, pages 1671-1679, abstract only.	1-44,49-54, 56-58
A,P	SPRECHER et al. Atrichia with papular lesions maps to 8p in the region containing the human hairless gene. American Journal of Medical Genetics. 28 December 1998, Vol 80, No. 5, pages 546-550, abstract only.	1-44,49-54,56-58

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle of theory underlying the invention
*A document defining the general state of the art which is not considered to be of particular relevance		
*E earlier document published on or after the international filing date	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O document referring to an oral disclosure, use, exhibition or other means		
*P document published prior to the international filing date but later than the priority date claimed	*A	document member of the same patent family

Date of the actual completion of the international search

12 MARCH 1999

Date of mailing of the international search report

12 APR 1999

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

RICHARD SCHNIZER

Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/02128

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 60-62  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
  
These claims are drawn to the antibody of claim 54. No antibody is recited in claim 54. If these claims are meant to be dependent on claim 59, then they would be improperly multiply dependent.
3. ☒ Claims Nos.: 45-48,55,59,63,64  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/02128

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-32 drawn to nucleic acids encoding part or all of the human hairless protein (hhp), vectors carrying the nucleic acid, host/vector combinations for expressing the gene, wild type and mutant human hairless proteins, and a first method of use of the claimed nucleic acids in isolating human hairless-related proteins.

Group II 33-38, drawn to methods of identifying compounds capable of enhancing or inhibiting expression of the hhp, and to any compound so identified.

Group III, claim(s) 39 and 40, drawn to a method for identifying a compound which binds to hhp, and to any compound so identified.

Group IV, claim(s) 41 and 42, drawn to a method of identifying a compound which inhibits the binding interaction of hhp with other compounds, and to any novel compound so identified.

Group V, claim(s) 43 and 44, drawn to transgenic animals comprising a wild type or mutant hhp gene.

Group VI, claim(s) 49-51, drawn to a transgenic animal knockout of the hhp transgene.

Group VII, claim(s) 52-54, 56 and 58 drawn to a method for identifying a compound which restores a normal phenotype to an animal model for human hairlessness, to any identified compound, and to pharmaceutical compositions for the delivery of the compound.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of group I is a novel isolated nucleic acid encoding part or all of the human hairless protein. The methods of groups II-IV and VII do not result in the isolation of this gene or related genes, and the isolated gene of group I does not directly yield the products or results of the methods of groups II-IV and VII. Neither is the nucleic acid of group I the transgenic animals of groups V or VI.

Therefore, the technical relationship among those inventions involving one or more of the same or corresponding technical features are not completely shared by each of the above indicated groups and do not define a contribution which each of the claimed inventions, considered as a whole, makes over the art.

